University of Alberta

Alcohol Dehydrogenase Catalyzed Diels-Alder Reactions and Synthetic Studies Toward Putative Intermediates in the Post-PKS Transformations of Dihydromonacolin L

by

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I have not set off this little work with pompous phrases, nor filled it with high-sounding and magnificent words, nor with any other allurements or extrinsic embellishments with which many are wont to write and adorn their works; for I wished that mine should derive credit only from the truth of the matter, and that the importance of the subject should make it acceptable.

Niccoló Machiavelli,
to the Magnificent Lorenzo,
son of Piero de' Medici

ABSTRACT

The ability of three different alcohol dehydrogenase enzymes (yeast alcohol dehydrogenase, horse liver alcohol dehydrogenase and hydroxysteroid dehydrogenase) to activate a dienophile by oxidation and catalyze an intramolecular Diels-Alder reaction was explored. To this end four different substrates ((2*E*,7*E*)-deca-2,7,9-decatrien-1-ol (**28**), (2*E*,7*E*,9*E*)-undeca-2,7,9-trien-1-ol (**29**), (2*E*,8*E*,10*E*)-dodeca-2,8,10-trien-1-ol (**30**) and (2*E*,9*E*,11*E*)-trideca-2,9,11-trien-1-ol (**31**)) were synthesized which featured a dienophile (allylic alcohol) tethered to a diene. All three enzymes were able to activate the dienophile by oxidation to the corresponding aldehyde although the oxidation was very slow (3 to 5 days at room temperature) and low yielding (<5%). However, the Diels-Alder reaction occurred in solution rather than in the active site of the enzyme.

During the course of this study, it was discovered that *o*-iodoxybenzoic acid (IBX) is able to act as both an oxidant and as a Lewis acid for the Diels-Alder reaction. The IBX oxidized undecatrienol **29** and dodecatrienol **30** to the corresponding aldehyde. The Diels-Alder reaction which ensued produced the *endo* stereoisomers (racemic) with high diastereoselectivity (indene aldehyde and decalin aldehyde).

A new lactone precursor, dimethyl (*R*)-(*tert*-butyldimethylsilyloxy)-2,6-dioxo-6-((S)-1-phenylethylamino)hexylphosphonate (**70**), was synthesized by using a modification of a literature route. This reagent was utilized in a Horner-Emmons-Wadsworth reaction with decalin aldehyde **79** and 6-Me-decalin aldehyde **78**. In addition, a labeled version, $^{13/14}$C-amide phosphonate **76**, was constructed and was unstable. It had a half life of two weeks at -20 °C. The synthesis of unlabeled desmethyl DHL **77** analog was pursued. However, in the last step of the synthesis to form the lactone, elimination of the C3 hydroxyl group occurred and provided the α,β-unsaturated compound **82**.

Finally, two short synthetic routes were discovered which provide access to monacolin L (**21**) and 3α-3,5-hydroxy-DHL **20**. The Hendrickson reagent was used to dehydrate TBDPS epoxides **92**(β) and **93**(α) which provided TBDPSmonL **95**. The Lewis acid aluminum isopropoxide was employed to rearrange TBDPS epoxides **92**(β) and **93**(α) and yielded 3β-3,5-hydroxy-DHL **96**. While the aluminum isopropoxide reaction was very low yielding and provided the allylic alcohol with the opposite stereochemistry desired, it is possible that the alcohol of 3β-3,5-hydroxy-DHL **96** could be inverted to form 3α-3,5-hydroxy-DHL **20** after removal of the protecting group.

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LIST OF ABBREVIATIONS

AcOH	acetic acid
pKa	acid dissociation constant
ACP	acyl carrier protein
AT	acyltransferase
1-ADNH$_2$	1-adamantamine
ADH	alcohol dehydrogenase
ACS	American Chemical Society
Ar	aryl
BMS	Bristol-Myers Squibb
calcd	calculated
^{13}C NMR	carbon-13 Nuclear Magnetic Resonance
CFE	cell free extract
CHS	chalcone synthase
CI	chemical ionization
δ	chemical shift recorded in parts per million
CoA	coenzyme A
Co	company
conc.	concentrated
M	molar concentration in moles per liter
c	concentration recorded in milligrams per deciliter

J	coupling constant recorded in hertz
DH	dehydratase
DEBS PKS	6-deoxy-erythronolide B polyketide synthase
DBU	1,8-diazabicyclo[5.4.0]-undec-7-ene
1,2-DCE	1,2-dichloroethane
DEAD	diethylazodicarboxylate
Et_2O	diethyl ether
ΔE	difference in energy
DHL	dihydromonacolin L
3,4-DHP	3,4-dihydropyran
DIBALH	diisobutylaluminum hydride
DIPEA	diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
dpm	disintegrations per minute
d	doublet
EI	electron impact
EWG	electron withdrawing group
ES	electrospray ionization
ER	enoylreductase
EtOH	ethanol
EtOAc	ethyl acetate

Et	ethyl group
FAD	flavin adenine dinucleotide (oxidized form)
FAS	fatty acid synthase
FT-IR	Fourier transform-infrared spectroscopy
GC-MS	gas chromatography-mass spectrometry
$t_{1/2}$	half life
h	heptet
HDL	high density lipoprotein
HOMO	highest occupied molecular orbital
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HEW	Horner-Emmons-Wadsworth reaction
HLADH	horse liver alcohol dehydrogenase
HMG-CoA Reductase	hydroxymethylglutaryl-coenzyme A reductase
HMG-CoA	hydroxymethylglutarate
SDH	hydroxysteroid dehydrogenase from *Pseudomonas testosteroni* (ATCC 11996)
C_2 units	intact structure composed of two carbons
*i*PrMgCl	isopropylmagnesium chloride
KR	keto reductase
KS	keto synthase
kDa	kilo Daltons

pH	logarithmic scale of the proton concentration in aqueous solution
LNKS	lovastatin nonaketide synthase
LDL	low density lipoprotein
LRMS	low resolution mass spectrometry
LUMO	lowest unoccupied molecular orbital
MPS	macrophomate synthase
MHz	mega hertz
MΩ	mega ohms
mp	melting point
m-CPBA	*meta*-chloroperoxybenzoic acid
$CH_3OB(Et)_2$	methoxydiethylborane
MsCl	methanesulfonyl chloride
Me	methyl
MeT	methyl transferase
mg/dL	milligrams per deciliter
mol	mole
M^+	molecular ion
m	multiplet
n-$BuNH_2$	n-butylamine
n-BuNH	n-butylamine group

$NADP^+$	nicotinamide adenine dinucleotide phosphate (oxidized form)
NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)
NMR	nuclear magnetic resonance
ORF	open reading frame
$[\alpha]_D^{25}$	optical rotation
IBX	*ortho*-iodoxybenzoic acid
[O]	oxidation reaction
p-TsO	*para*-toluenesulfonate
p-TsOH	*para*-toluenesulfonic acid
%	percentage
Ph	phenyl group
PKS	polyketide synthase
PSI	pounds per square inch
^{1}H COSY	proton shift correlation spectroscopy
^{1}H NMR	proton nuclear magnetic resonance
quant.	quantitative
q	quartet
R_f	retention factor
RT	room temperature
rpm	rotations per minute

SAM	S-adenosyl methionine
sat.	saturated
SiO_2	silica gel
s	singlet
NaOAc	sodium acetate
TBDMSO or OTBDMS	*tert*-butyldimethylsilyloxy group
TBDMS	*tert*-butyldimethylsilyl group
TBDPSCl	*tert*-butyldiphenylsilyl chloride
TBDPS	*tert*-butyldiphenylsilyl group
TBDPSO	*tert*-butyldiphenylsilyloxy group
THF	tetrahydrofuran
THL	tetrahydromonacolin L
TBAF	tetra-*n*-butylammonium fluoride
OTHP	tetrahydro-2*H*-pyranyl ether
TMS	tetramethylsilane
TMS-Cl	tetramethylsilylchloride
TLC	thin layer chromatography
TE	thioesterase
*t*RNA	transfer ribonucleic acid
NEt_3	triethylamine
Et_3SiH	triethylsilane
TFA	trifluoroacetic acid

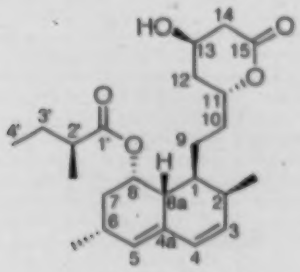
$(TfO)_2O$	trifluoromethanesulfonic anhydride
UV	ultraviolet
YADH	yeast alcohol dehydrogenase

CHAPTER 1. Introduction

1.1 Lovastatin and the Statin Drugs

In the United States, approximately 105.2 million adults have blood cholesterol levels considered to be borderline high risk (200-239 mg/dL)[1] of which nearly 36.6 million adults have cholesterol levels considered to be high risk (>240 mg/dL).[1] Perhaps it is no surprise that in 2005 the total global sales for cholesterol and triglyceride reducers was 32.6 billion US dollars which meant that they are the top selling class of drugs.[2]

Figure 1 Structure of lovastatin (**1**)

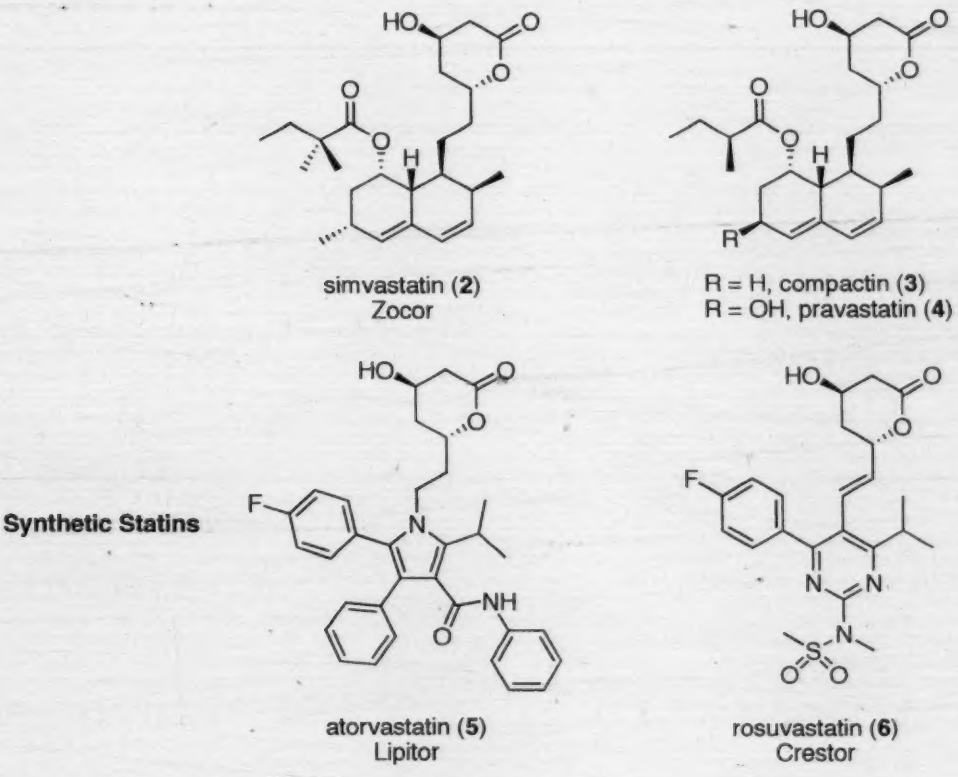


lovastatin (**1**)

Lovastatin (**1**) is a fungal polyketide which was first isolated in 1979 from *Monascus ruber* by Endo and Hasumi and shortly after from *Aspergillus terreus* by Merck researchers in 1980 (Figure 1).[3, 4] It was discovered to be an anti-fungal compound as well as a potent, slow-binding inhibitor of

hydroxymethylglutaryl-coenzyme A reductase (HMG-CoA reductase), which catalyzes the rate-limiting step in cholesterol biosynthesis (Scheme 1). Compactin (**3**), also a fungal polyketide and slow-binding inhibitor of HMG-CoA reductase, was isolated in 1976 from *Penicilium citrinum* (Figure 2).[5] The only difference in structure between compactin (**3**) and lovastatin (**1**) is the methyl group at C6 (missing in compactin (**3**), see Figure 2). By adding an additional methyl group at the 2' position of lovastatin (**1**), Merck created its cholesterol lowering drug Zocor (**2**).[6] A microbial hydroxylation of compactin (**3**) was used to stereoselectively introduce the hydroxyl group at C6 in pravastatin (**4**).[7, 8] In addition to these two semi-synthetic cholesterol lowering drugs, there are a number of important synthetic cholesterol lowering drugs, including Lipitor (**5**, atorvastatin) and Crestor (**6**, rosuvastatin) (Figure 2). The top-selling drug in the world is Lipitor (**5**) with sales of 12.9 billion US dollars in 2005, more than twice the sales of Zocor (**2**).[2]

Figure 2 Structures of selected cholesterol-lowering drugs

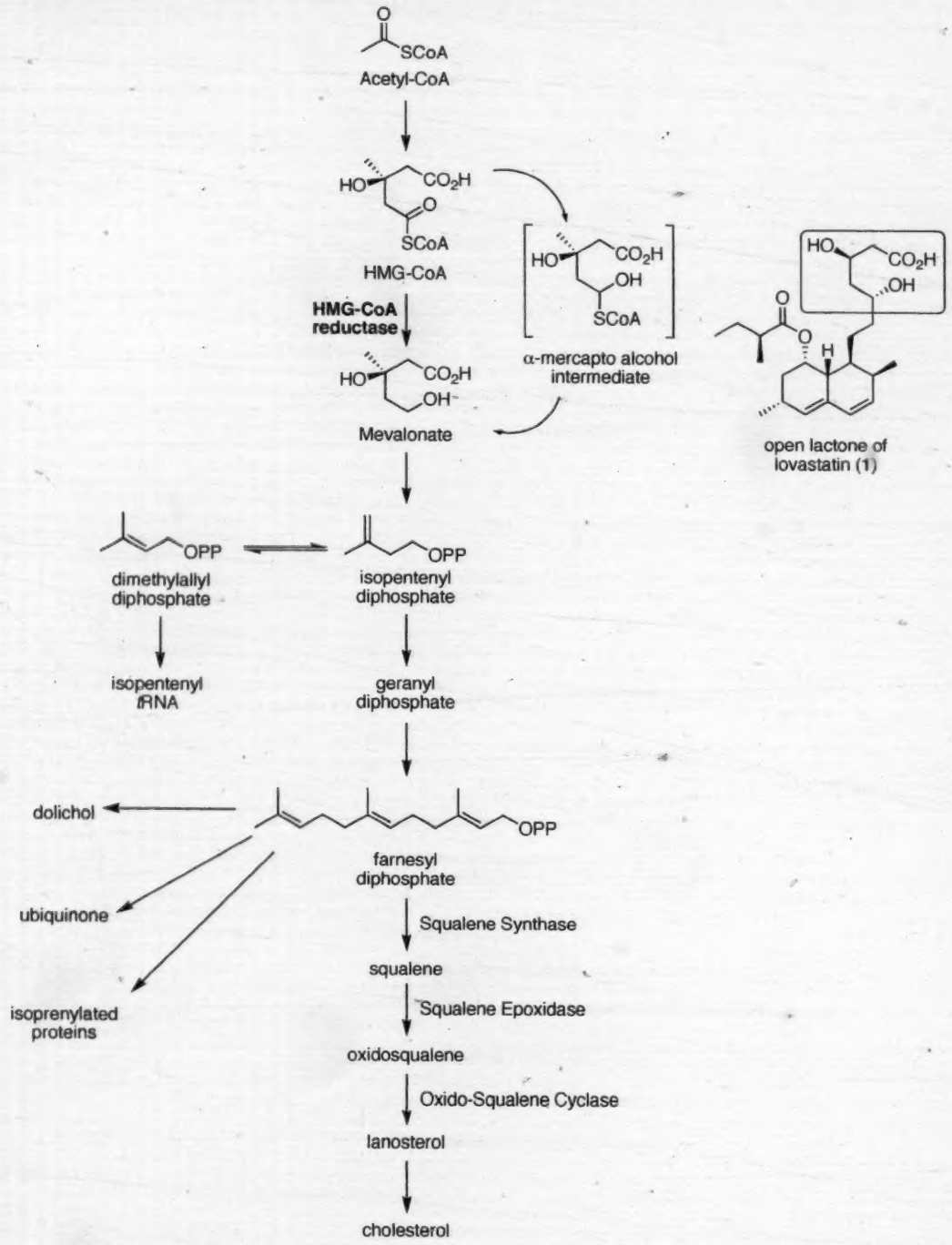


1.1.1 Mode of Action

To understand how the cholesterol-lowering statin drugs exert such a dramatic effect on cholesterol levels in the human body requires consideration of two facts. Biosynthesis of cholesterol from acetyl-CoA in the liver accounts for 60-70% of the total cholesterol pool in the body.[9] In addition, the enzyme that is inhibited by the statin drugs, HMG-CoA reductase, catalyzes the rate-limiting step in the

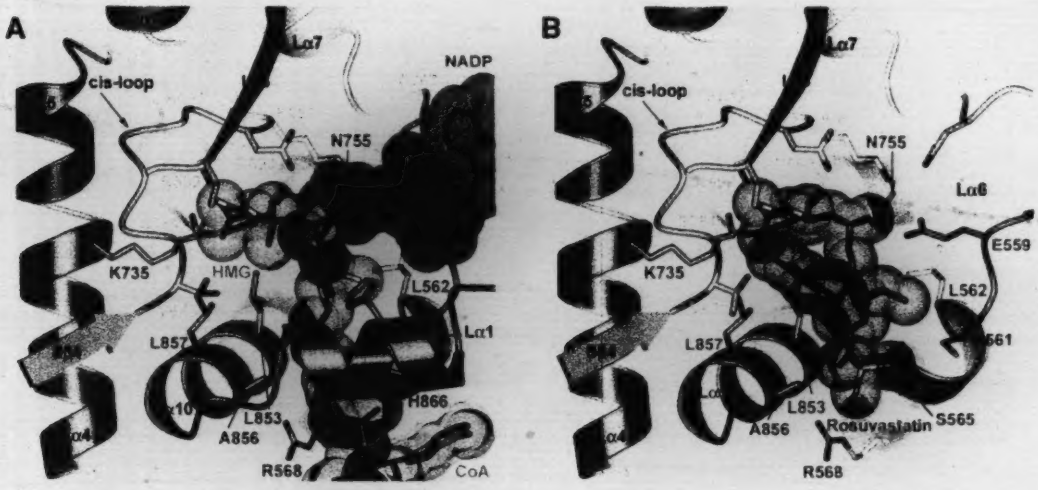
biosynthesis of cholesterol (Scheme 1). This enzyme is quite interesting in that it utilizes two molecules of reduced nicotinamide adenine dinucleotide phosphate (NADPH), sequentially to reduce HMG-CoA to mevalonate via an α-mercapto alcohol intermediate. Despite the structural diversity of the statin drugs, they bind to HMG-CoA reductase in a similar manner. All of them require a mevalonate like moiety to mimic the α-mercapto alcohol intermediate in order to bind to the enzyme.[10]

Scheme 1 Biosynthesis of cholesterol highlighting the mechanism of HMG-CoA reductase



The statin drugs (prodrugs) are often administered as the inactive lactone.[11] The lactone is hydrolyzed in the liver to the carboxylate salt, and it is believed that it is this form that binds to the enzyme.[10] The differences in inhibition between the drugs arises from the difference in their abilities to interact with an adjacent hydrophobic binding pocket in the enzyme. Shown in Figure 3 are the crystal structures of the human HMG-CoA reductase active site with hydroxymethylglutarate (HMG), CoA, $NADP^+$ and with the inhibitor rosuvastatin (**6**) bound (Figure 3 **A** and **B** respectively).

Figure 3 X-ray crystal structure of the active site of human HMG-CoA reductase. **A** Shows the natural substrate, hydroxymethylglutarate, bound along with $NADP^+$ and Coenzyme A. **B** Shows the X-ray crystal structure of the inhibitor rosuvastatin (**6**)(taken with permission from Istvan *et al*)[10]



The active site is formed at the interface of two monomers. One monomer is shown in yellow the other in aqua (Figure 3). Shown in purple in the left hand

structure are HMG and CoA. The co-factor, $NADP^+$ is shown in green. As expected the mevalonate moiety of the inhibitor binds in the HMG binding pocket. The part of the enzyme which is reorganized upon binding of the inhibitor to the enzyme is helix Lα11. This is displaced to reveal a shallow hydrophobic groove which serves as the phosphopantethienyl binding region with the native substrate.[10] The significant and surprising result of this study was the discovery that the inhibitors bind to the phosphopantetheinyl region of the enzyme.[10]

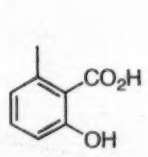
In addition to the primary mode of action of the statin drugs (inhibition of HMG-CoA) it has been found that this class of compounds have a myriad of other beneficial effects on the human body. They were found to lower the blood concentration of low density lipoprotein (LDL) which is a risk factor for premature coronary heart disease.[12, 13] Conversely, they have been found to increase the concentration of beneficial high density lipoprotein (HDL) in the blood.[13] The statin drugs were found to have potent anti-inflammatory activity which is independent of their ability to lower cholesterol.[14] A recently published clinical study provides the first strong evidence that a statin drug can reduce arterial plaque build-up (atherosclerosis).[15] As well, there is evidence that statins can prevent the formation of β-amyloid plaques associated with Alzheimers disease.[16, 17] The main side effect of statin therapy is muscle degradation (rhabdomyolysis), a condition that received much attention due to the recall of Baycol (cerivastatin) in 2001. However, in a study published in 2004, out of a

patient pool of 252 460, only 24 individuals were hospitalized due to development of this severe side effect as a result of statin therapy.[18] Thus, it is clear that the benefits of statin therapy far outweigh any negative side effects.

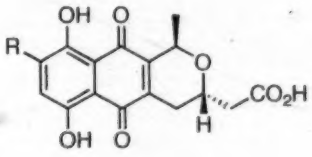
1.2 Polyketide Natural Products

Polyketides are a class of natural products with a wide variety of structures and biological activities. Lovastatin (**1**), as previously mentioned, belongs to this class of compounds and shown in Figure 4 are representative members of this group. The relatively simple aromatic antibiotic 6-methylsalicylic acid (**7**) was the subject of Arthur Birch's landmark study which will be discussed in a later section.[19] Actinorhodin (**8**) is a beautiful blue compound with antibiotic activity.[20] The extremely carcinogenic aflatoxin B_1 (**9**) is a polyketide made from a fungus which is known to thrive on peanuts.[20] Macrolide antibiotics are represented by the well studied erythromycin A (**10**).[20] Finally, the complex polyene amphotericin B (**11**) is an interesting example of a polyketide natural product with antifungal activity.[21] Despite their range of structural complexity, all of the members of this class of natural products are constructed from simple carboxylic acid monomers using a set of similar enzymatic processes.

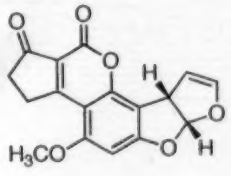
Figure 4 Selected examples of well known polyketide natural products



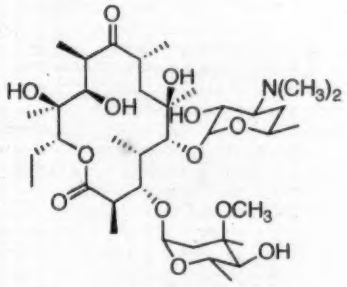
6-Methylsalicylic acid (**7**)



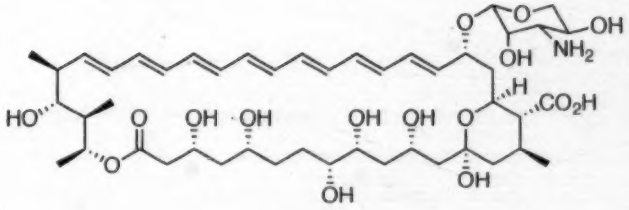
Actinorhodin (**8**)



Aflatoxin B_1 (**9**)



Erythromycin A (**10**)



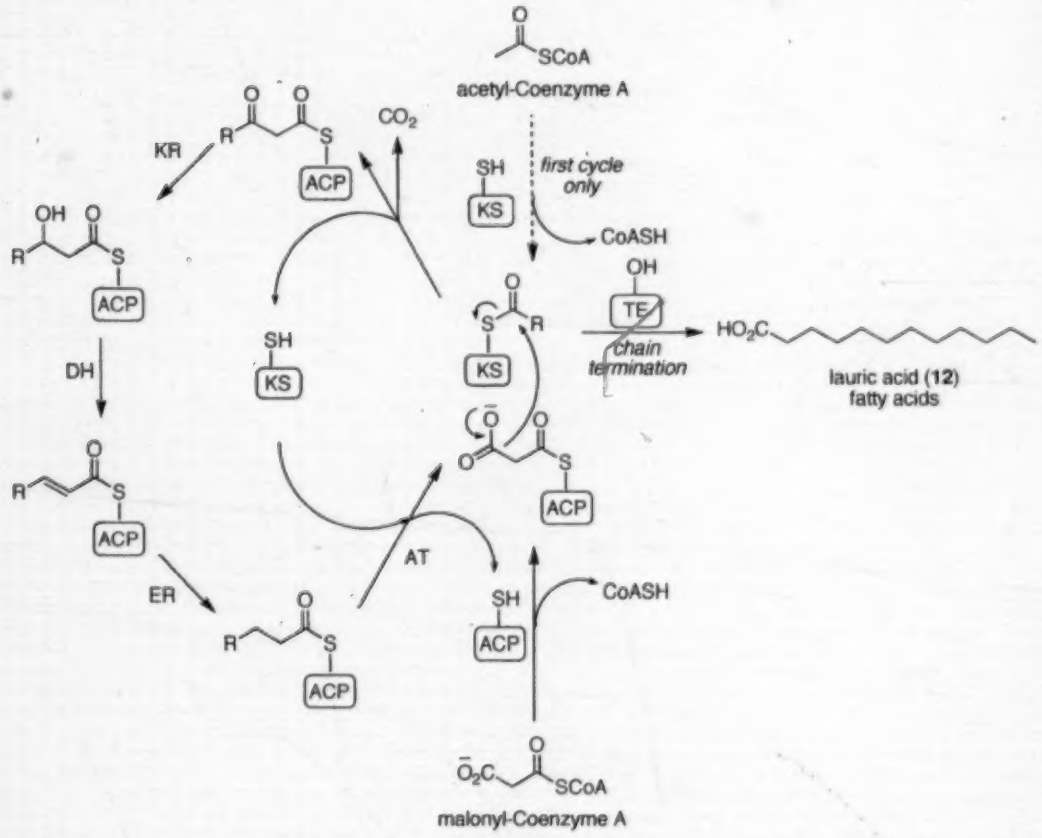
Amphotericin B (**11**)

1.2.1 Fatty Acid Biosynthesis Versus Polyketide Biosynthesis

Fatty acids are assembled by a head to tail attachment of C_2 units.[22, 23] The biosynthesis usually starts with the loading of acetyl-CoA (starter unit) onto the keto synthase (KS) of the Fatty Acid Synthase enzyme (FAS) as a thioester. The starter unit is then condensed with a malonate unit attached to an acyl carrier protein (ACP) via a Claisen condensation with concommittent loss of carbon dioxide (Scheme 2). However, the participating thiol is not a part of the primary protein sequence, but rather it is from the phosphopantetheinyl unit added to the ACP protein by post-translational modification.[22, 23] The β-keto-thioester which is

formed then gets reduced by the keto-reductase (KR) to a secondary alcohol. The alcohol is then removed by the dehydratase (DH) to form an α,β-unsaturated thioester. Finally, the double bond is reduced by the enoyl reductase (ER) to give the aliphatic thioester. This cycle repeats by transferal of the growing chain back to the KS and then subsequent condensation with another unit of malonate (Scheme 2).

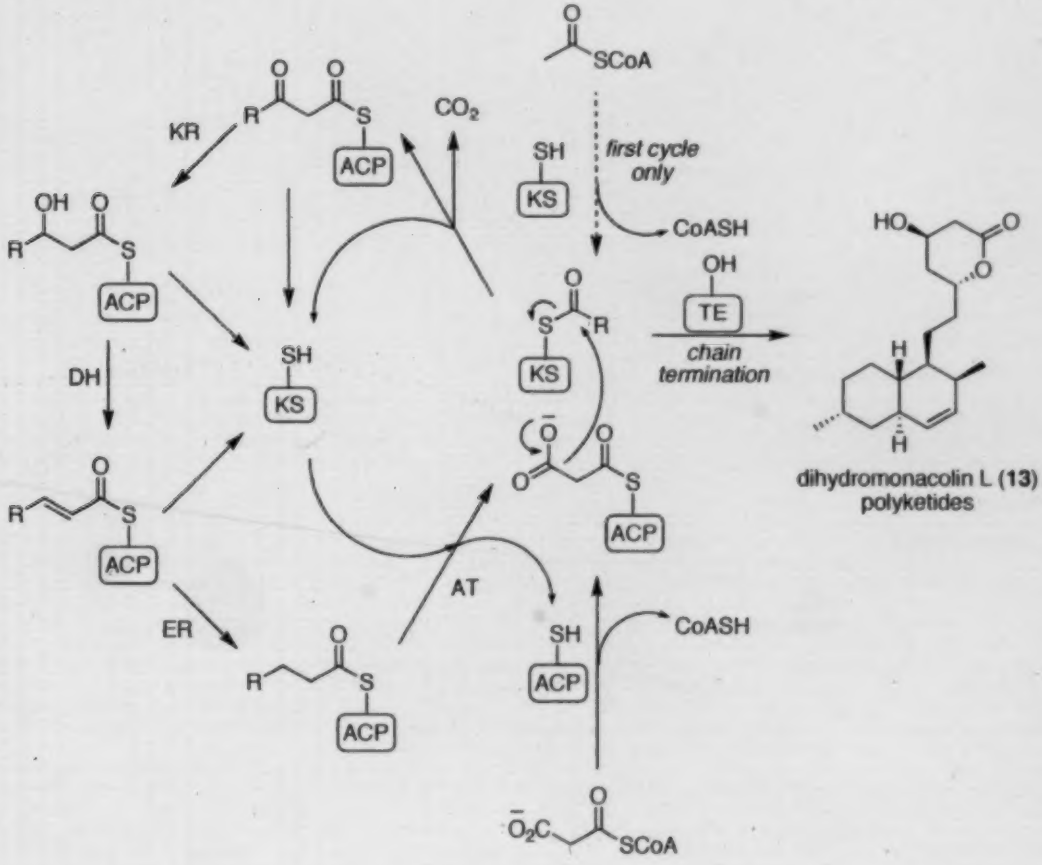
Scheme 2 Illustration of the chemical reactions involved in the biosynthesis of fatty acids



When the fatty acid chain reaches the desired length the cycle is terminated by transferring the fatty acid to the thioesterase (TE). The fatty acid is then released as the acid or as an acyl ester (Scheme 2). For example, lauric acid (**12**) is formed by one molecule of acteyl-CoA and five molecules (five cycles) of malonyl-CoA before release. The FAS enzymes all have the same set of enzyme activites (KS, ACP, KR, DH, ER, AT and TE) and the cycle shown in Scheme 2 is not interrupted at intermediate stages to produce compounds with

varied oxidation levels. This is not the case with polyketide synthase enzymes (PKS). The minimal PKS is comprised of four active sites (ACP, KS, AT and TE) each within separate domains.[20] The remaining three enzyme activities KR, DH and ER are used variably by the PKS enzyme to yield products with a range of oxidation states, in contrast to the fully reduced products made by FAS. This variable use is illustrated in Scheme 3 by the red arrows. The loading and condensation by a Claisen type reaction is the same as for FAS. However, once the β-keto-thioester intermediate is formed, the PKS can interrupt the cycle at intermediate stages to leave a ketone, alcohol or double bond intact prior to extension with another C_2 unit. Thus, the range of compounds formed can be very complex as is illustrated by dihydromonacolin L (**13**) (Scheme 3). An additional variation from FAS by PKS is the way in which the product is released from the enzyme. Similar to FAS the final polyketide chain can be transferred to the TE domain, but for PKS the product is released by either intermolecular attack by water (forms a free acid) or by intramolecular attack by a free hydroxyl (forms a lactone).[20]

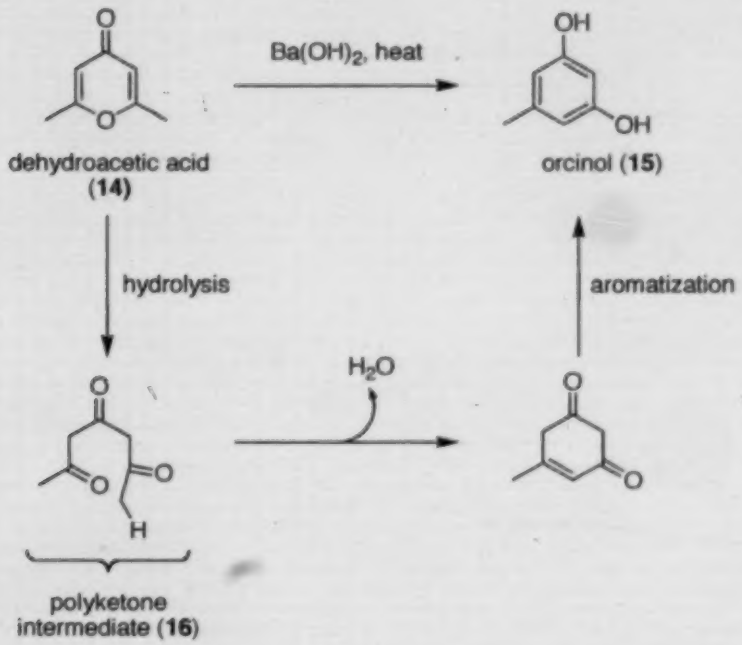
Scheme 3 Illustration of the chemical reactions involved in the biosynthesis of polyketides



1.2.2 A Brief History of Polyketide Research

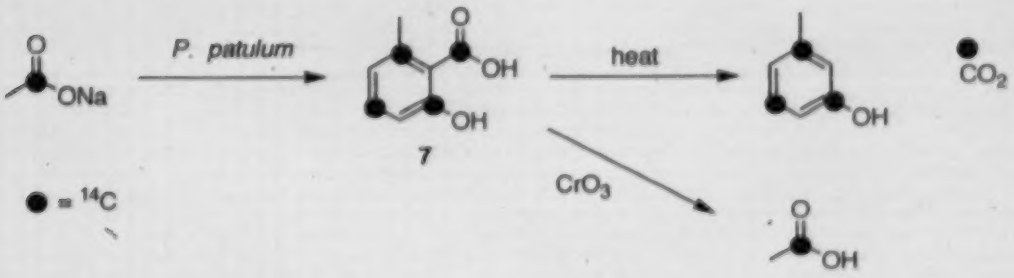
In 1893 James Collie was attempting to establish the structure of dehydroacetic acid (**14**) by degradation with aqueous barium hydroxide ($BaOH_2$). He discovered that an aromatic compound was one of the products (Scheme 4). Subsequently, he was able to deduce the structure of the aromatic product as orcinol (**15**). Based on his proposed mechanism for the formation of **15**, he made the even more daring prediction that other natural polyphenols could be constructed from polyketone intermediates such as **16**.[24]

Scheme 4 Collie's proposed mechanism for the formation of orcinol (**15**) from dehydroacetic acid (**14**)



It would be another 50 years before someone would put Collie's hypothesis to the test. Sir Robert Robinson mentioned in his book[25] the possibility that aromatic phenols could be formed from polyketones and reinforced the validity of James Collie's hypothesis, but it would be his student who would do the hard work. Arthur Birch was the person who gave the field of polyketide research its start. His molecule of study was 6-methylsalicylic acid (**7**) produced by the fungus *Penicillium patulum*.[19, 26] To test Collie's hypothesis, Prof. Birch fed radioactively labeled sodium acetate to *P. patulum*, then determined the locations of the radioactive atoms in **7** by extensive degradation analysis (Scheme 5).[19]

Scheme 5 Experimental results of both incorporation of 1-[^{14}C]-sodium acetate by *P. patulum* to make 6-methylsalicylic acid (**7**) and the degradation analysis to locate the radioisotope labels



The experimental labeling pattern was found to be consistent with the head to tail attachment of acetates predicted by Collie's hypothesis (Scheme 5).[19] This was the first landmark study that proved Collie's ideas were correct. The major drawback of this method is the analysis by degradation. This labor intensive

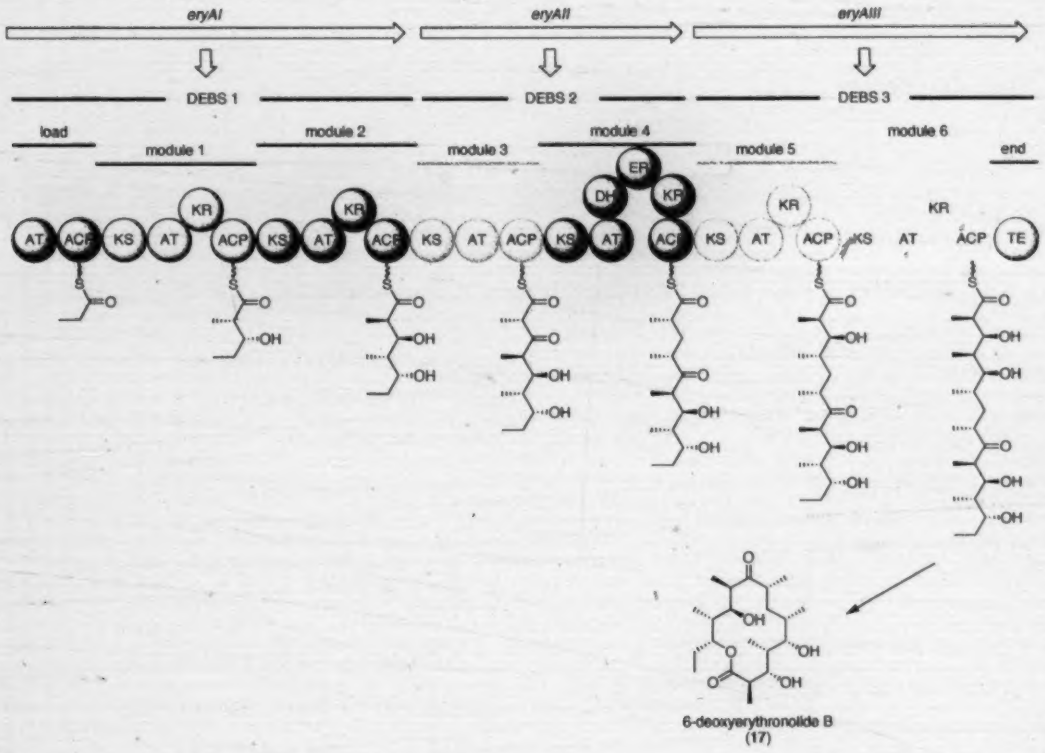
procedure could take years to complete in difficult cases. However, the invention of Nuclear Magnetic Resonance (NMR) and the realization that carbon-13 could be visualized by this method[27] heralded a new era of natural products research. The first demonstration of incorporation of carbon-13 labeled sodium acetate for biosynthetic studies was in the biosynthesis of griseofulvin.[28] The use of NMR to follow the fates of atoms in biosynthesis was more efficient than radioactive labeling and was proven to be useful in other natural products.[29, 30] Thus, researchers could study labeling patterns using stable nuclei such as hydrogen, deuterium and carbon.[31] What these methods could not shed light on is how the polyketide synthase enzymes can make such a wide variety of structures from relatively few chemical reactions. It became apparent that it was necessary to study the enzymes' structures and active sites to gain a clearer understanding of what was happening during the biosynthetic steps.

The first polyketide biosynthetic gene cluster to be sequenced was the one which produced actinorhodin (**8**) from *Streptomyces coelicolor*.[32] In this pioneering work Hopwood and coworkers were able to demonstrate that the biosynthetic genes are clustered together in the genome. They also developed the genetic methods (heterologous expression) that served to inspire other researchers in the field.[32] The system which has proven to be most amenable to genetic analysis and manipulation is the 6-deoxy-erythronolide B polyketide synthase (DEBS PKS).[33, 34] This is a modular Type I PKS system which fortuitously has all of the active

sites on discrete domains arranged in modules (Figure 5). Each of the active sites is used only once during the biosynthetic sequence. Therefore one can predict the structure of the polyketide (in this case 6-deoxyerythronolide B (**17**)) product based on the peptide sequence.

Two representative examples of the many methods of genetic manipulation of the DEBS PKS gene cluster to generate novel natural products are domain mutagenesis and combinatorial biosynthesis.[20, 35] In the domain mutagenesis approach one of the domains of the gene cluster is inactivated or swapped.[20] In the combinatorial biosynthesis method two or more PKS genes from the same or different origins are ligated together.[35]

Figure 5 Schematic representation of the organization of DEBS PKS and the biosynthesis of 6-deoxyerythronolide B (**17**)(taken with permission from Staunton 2001)[20]



1.2.3 Classification of Polyketide Synthases

According to current conventions the PKS enzymes are divided into several classes according to the arrangement and use of their active sites (Table 1).[36] However, it should be noted that due to recent discoveries, this convention is presently contested in the literature.[36, 37]

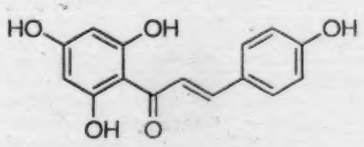
Table 1 A summary of the different types of PKS enzymes and their origins

PKS Type	Origin	Product	Source
I *modular*	bacterial	erythromycin A (**10**)	*Saccharopolyspora erythraea*
I *iterative*	fungal	6-methylsalicylic acid (**7**)	*Penicillium patulum*
		lovastatin (**1**)	*M. ruber* and *A. terreus*
II *iterative*	bacterial	actinorhodin (**8**)	*Streptomyces coelicolor*
III	plant	chalcone (**18**)	many higher plant species

The modular Type I PKSs are found in bacteria and other microbes. The well studied PKS system which creates erythromycin A was isolated from *S. erythraea*. This type of PKS has all of the active sites arranged in sequential order on a large polypeptide chain. Each of the active sites is used only once to assemble the polyketide. It is thus possible in many cases to predict the final

poyketide structure based on the protein sequence. The iterative Type I PKS is typified by the PKS for lovastatin (**1**) and 6-methylsalicylic acid (**7**). These PKS systems are found in fungi and have one active site for each catalytic function. Unlike the modular Type I PKS, these active sites are used repeatedly to build the polyketide chain. The factors which govern when and how these active sites are used remain unknown. It is for this reason that the final product structure cannot be predicted based on the gene sequence for this type of PKS. The products of iterative Type II PKS enzymes are aromatic phenols with similar structures to that of actinorhodin (**8**)(Figure 4). The arrangement and use of active sites is similar to that of the iterative Type I PKS (Figure 5).

Figure 6 Structure of chalcone (**18**) with the starter unit highlighted in red



chalcone (**18**)

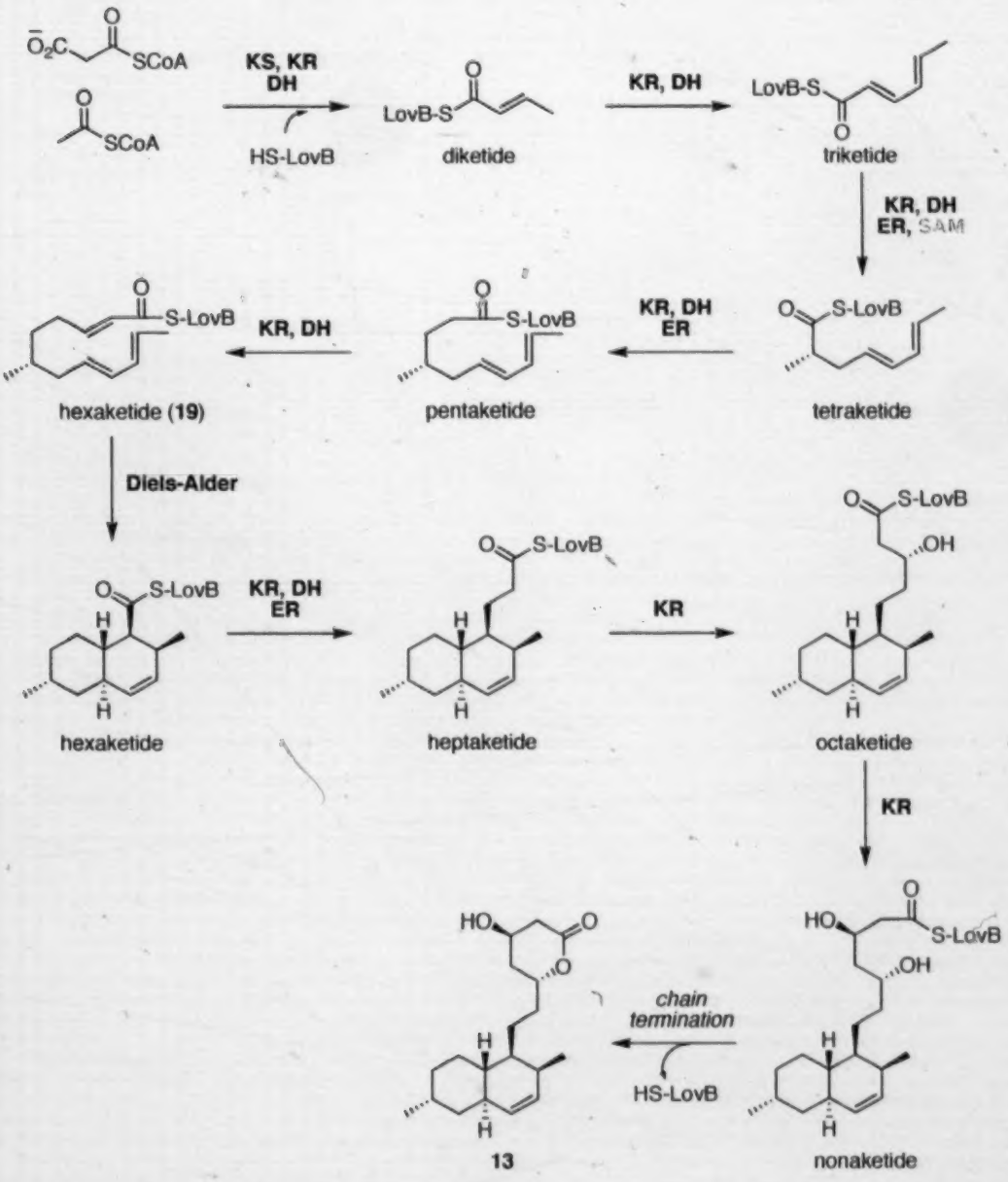
The most well studied Type III PKS are the chalcone synthases (CHS) which produce compounds similar to **18** (Figure 6). This type of PKS is very different from all known PKS and FAS systems. The CHS enzyme utilizes a *p*-coumaryl-CoA starter unit (highlighted in red, Figure 6) for the synthesis of chalcone

instead of acetyl-CoA like the other PKS enzymes. In addition the Type III enzymes directly utilize CoA thioesters to send intermediates to the different active sites rather than the phosphopantetheine arm of ACP. However, the most striking difference is that the Type III PKS carries out a series of decarboxylation, condensation, cyclization and aromatization reactions using only one active site.[20, 38]

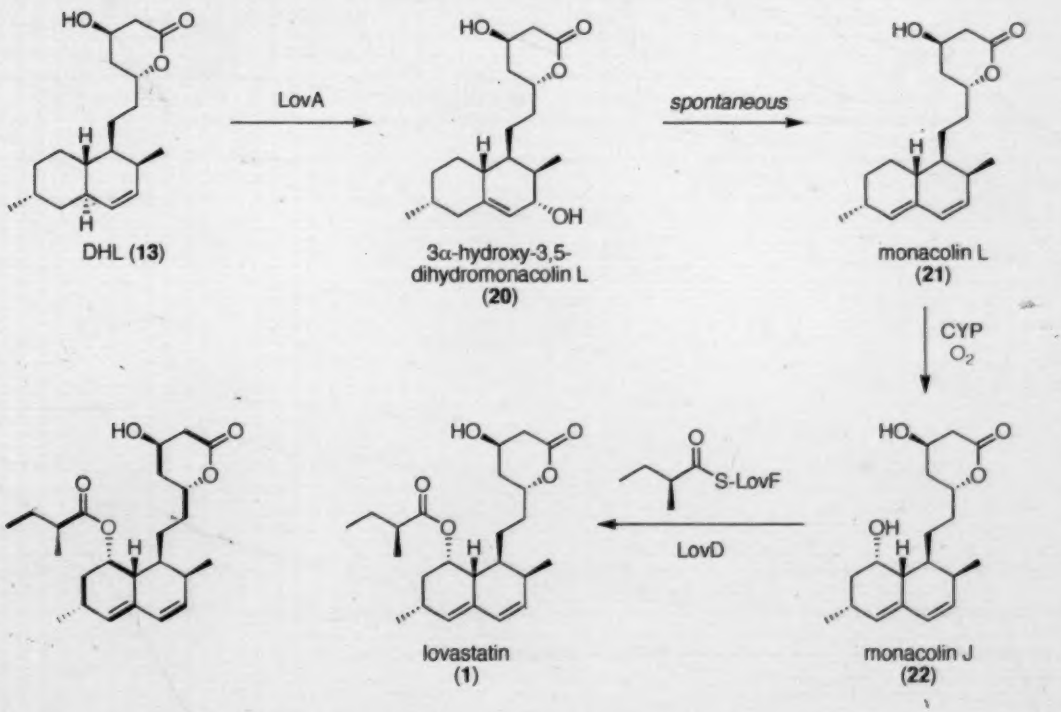
1.3 Biosynthesis of Lovastatin (1)

It was envisioned that lovastatin (**1**) was constructed in two main stages, *assembly* and *elaboration*. Initially, the nonaketide fragment, dihydromonacolin L (DHL) **13**, could be *assembled* by a PKS or lovastatin nonaketide synthase (LNKS) (Scheme 6)[39] and then DHL **13** could be *elaborated* to lovastatin (**1**) by a series of oxidations to install the oxygen at C8 and the diene system, concluding with acylation of the C8 oxygen with the diketide fragment (Scheme 7). The proposed biosynthesis for the assembly of DHL **13** has two intriguing features. The first is the presence of the methyl group at C6 which orginates from S-adenosyl methionine (SAM, shown in orange in Scheme 6). The use of SAM is usually only seen in the fungal, iterative Type I PKS enzymes. The second key point in the assembly of **13** is the proposed Diels-Alder cyclization at the hexaketide **19** stage to construct the dihydronapthalene ring and four stereocenters in one step.[40]

Scheme 6 Proposed biosynthesis for the assembly of dihydromonacolin L (DHL, **13**) by lovastatin nonaketide synthase (LNKS or LovB) with LovC



Scheme 7 Proposed post-PKS biosynthesis for the elaboration of DHL **13** into lovastatin (**1**) which summarizes the origins of all the atoms



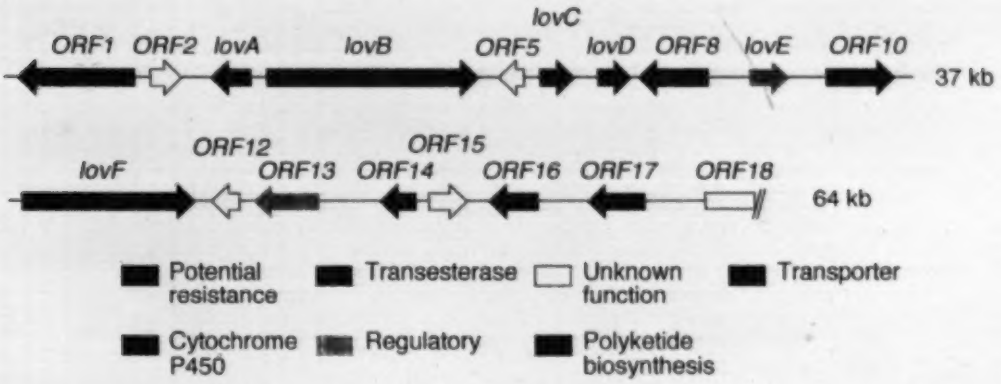
The elaboration of DHL **13** into lovastatin (**1**) illustrated in Scheme 7 was proposed in 1990 by Endo and co-workers.[41] It is thought that DHL **13** is oxidized to the allylic alcohol, 3α-hydroxy-3,5-dihydromonacolin L (**20**) by a cytochrome P450 enzyme. This intermediate is not stable and spontaneously undergoes dehydration to monacolin L (**21**). The C8 atom is oxidized stereospecifically by a cytochrome P450 to produce monacolin J (**22**). Finally, monacolin J (**22**) is esterified by a diketide to produce lovastatin (**1**). This biosynthetic pathway was studied by our laboratory in the early 1980's using a combination of stable isotope incorporation and NMR methods (summarized by the final structure in

color in Scheme 7).[42] In addition to the methyl group at C6, there is a methyl group on the side chain at C2' which originates from SAM (in orange in Scheme 7). Based on these results it was expected that there would be two different PKS enzymes in the biosynthetic gene cluster, a nonaketide PKS and a diketide PKS.

At this point further advancement in the field required the isolation and study of the proteins involved in the biosynthesis. Based on the pioneering genetic research of Reeves and McAda at Panlabs some of the genes involved in the biosynthesis of lovastatin (**1**) were identified.[43] They were proposed to be PKS genes based on sequence homology to other known PKS genes.

As the biosynthetic genes for secondary metabolites are usually clustered close together in the genome, it was then possible to identify all of the genes involved in the biosynthesis of lovastatin (**1**). This research was undertaken by Professor C. R. Hutchinson at the University of Wisconsin in close collaboration with our group. Professor Hutchinson was able to locate eighteen potential genes involved in the biosynthesis of lovastatin (**1**).[39] The functions for thirteen of the genes were assigned based on comparison with databases of known proteins and in some cases confirmed by functional mutation and isolation of the products (Figure 7).

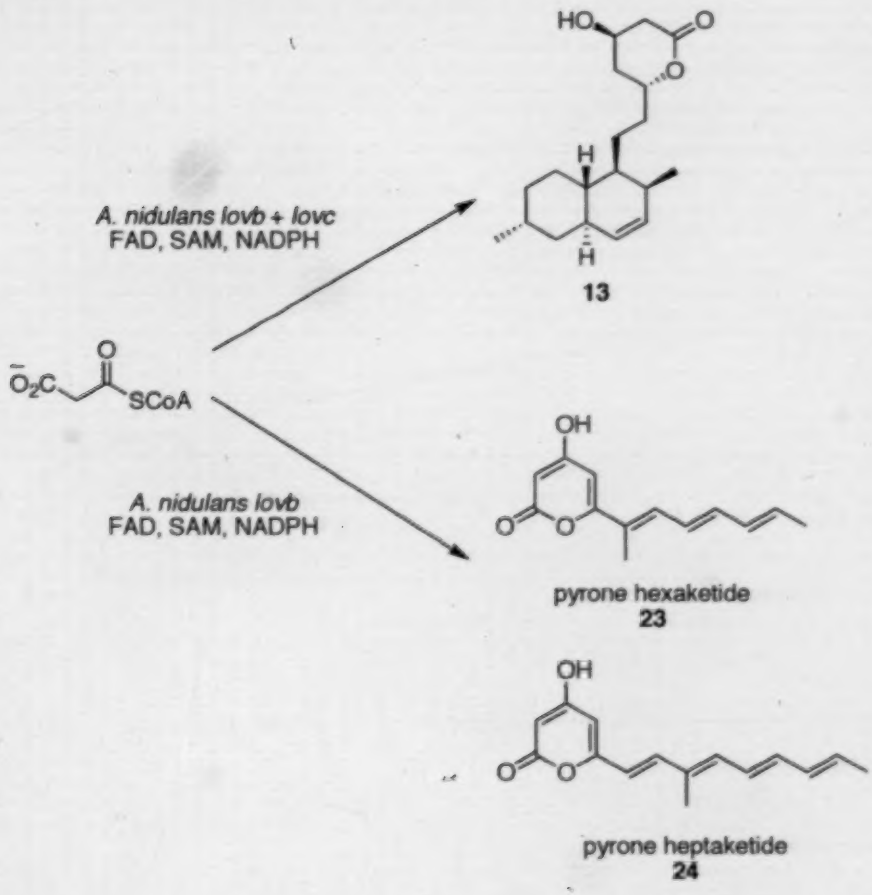
Figure 7 Lovastatin (**1**) biosynthetic gene cluster from *A. terreus*



There were two PKS gene sequences (*lovb* and *lovf*) in the cluster encoding proteins of the expected molecular weights of 335 kDa and 277 kDa, respectively (Figure 7). They both contained the six characteristic domains of PKS enzymes (KS, AT, ACP, KR, ER and DH) as well as an additional methyl transferase (MeT) domain. The gene sequence for *lovb* had some unusual characteristics. For one, there was no obvious TE domain, but rather a C-terminal condensation domain similar to a non-ribosomal peptide cyclization domain. In addition, the ER domain did not conform well to known sequences from PKS or FAS and was expected to be inactive. An esterase encoding gene (*lovd*) and two cytochrome P450 encoding sequences (*lova* and *ORF17*) were also identified (Figure 7). The role of the gene encoding *lovc* was unclear at this point. The remaining genes appeared to be involved in regulation, transport and self-resistance.

In order to verify that the assigned function of the genes was correct the PKS genes from *A. terreus* were expressed in a host fungus (heterologous expression), *Aspergillus nidulans*, which cannot produce lovastatin (**1**). It was found that when LovB and LovC were expressed together, DHL **13** could be isolated from the fermentation media.[39] However, when LovB was expressed by itself, **13** could not be isolated. Instead, two unstable pyrones, **23** and **24**, were isolated (Scheme 8). These shunt pyrones reveal that the LovB enzyme is apparently unable to reduce the double bond at the tetraketide stage as it normally does in the presence of LovC. In addition, the enzyme is unable to carry through the synthesis to the nonaketide stage and stops at both the hexaketide and heptaketide stages. Thus, LovC was deduced to be an accessory protein required for proper ER activity and necessary for the proper synthesis of the nonaketide chain. It was found that the esterification of C8 of monacolin J (**22**) with the diketide only occurred when both LovD and LovF were present. The proper reaction did not occur with LovF alone.[11, 39]

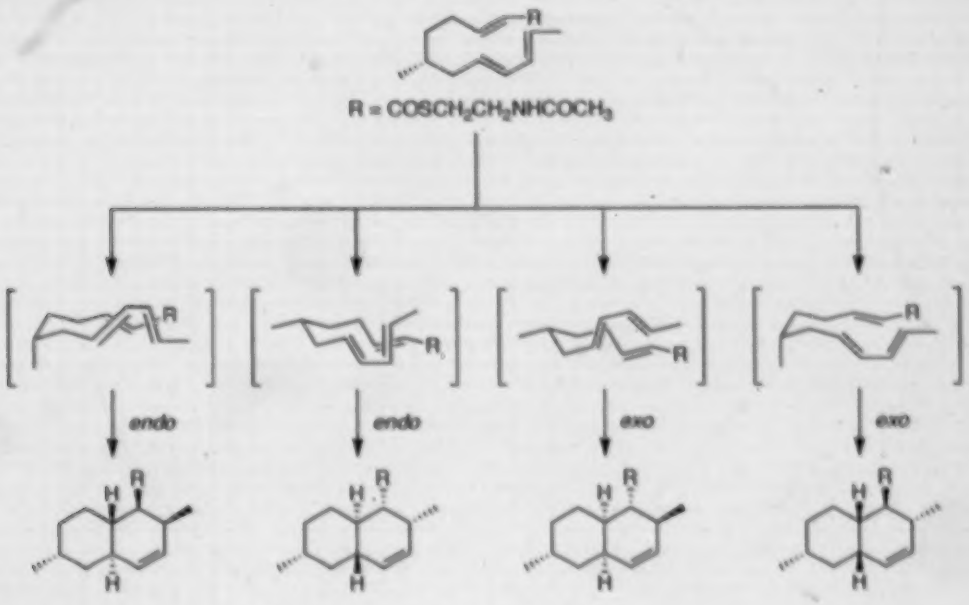
Scheme 8 Summary of the heterologous expression results of LovB and LovC together as well as LovB alone in *A. nidulans*



According to the biosynthetic hypothesis, the LovB enzyme would be expected to catalyze a Diels-Alder cyclization. To address this intriguing possibility, LovB protein was exposed to the N-acetylcysteamine thioester of the proposed hexaketide intermediate (R in Scheme 9). In the absence of the LovB protein only two products are formed (in a 1:1 ratio) as determined by GC-MS analysis (Scheme 9). These are the products with the C6 methyl group in a pseudo-

equatorial position. None of the product with the correct stereochemistry found in lovastatin (1) could be detected in the product mixture (Scheme 9). However, when the triene is allowed to cyclize in the presence of active LovB protein, the product with the correct stereochemistry could be detected, as well as the two products seen in the control experiment (ratio of 1:15:15 Scheme 9). Importantly, the product with the correct stereochemistry is not formed when the triene is allowed to cyclize in the presence of LovB protein which has been inactivated by heat. These results suggest that the LovB protein is responsible for catalyzing the Diels-Alder cyclization and thus represents the first example of a purified protein able to catalyze such a reaction.[39]

Scheme 9 *Experimental results for the enzyme catalyzed Diels-Alder activity of the LNKS enzyme*

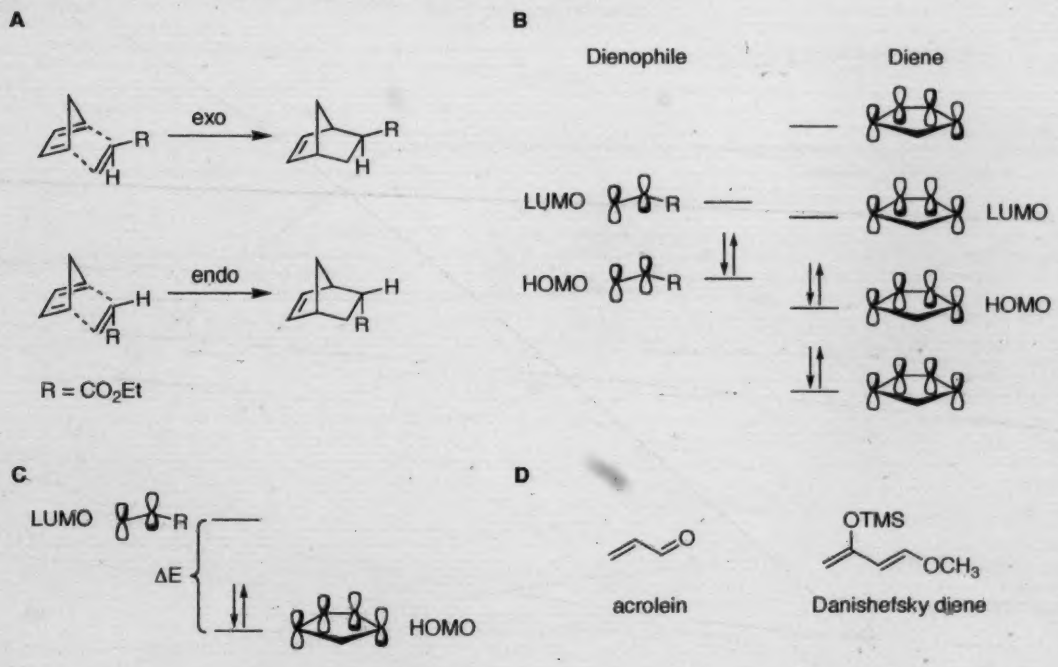


AQUEOUS		1	1
ENZYMATIC	1	15	15

1.4 The Diels-Alder Reaction

The Diels-Alder reaction, named after its discoverers Otto Diels and Kurt Alder who received the Nobel prize in 1950, is arguably one of the most useful chemical transformations that synthetic chemists have at their disposal. The reason for this is that it enables the formation of multiple rings and up to four stereogenic centers in one step. The use of the Diels-Alder reaction in total synthesis of numerous natural products is described extensively elsewhere.[44] The Diels-Alder reaction is a [4+2] cycloaddition reaction which features a concerted formation of two new bonds in the same transition state. This formation is not necessarily synchronous for all cases.[45]

Figure 8 A summary of the important aspects of the Diels-Alder reaction. **A** The difference between the *exo* and *endo* transition states. **B** The Frontier Molecular Orbitals of both the dienophile and diene. **C** The two Molecular Orbitals which govern the rate of the Diels-Alder reaction. **D** Examples of a reactive dienophile, acrolein, and a reactive diene, Danishefsky's diene.



The Diels-Alder reaction proceeds through two pathways termed the *exo* and *endo* pathways (Figure 8 **A**). Generally, the *endo* pathway is favored over the *exo* pathway. It is thought that the reason behind this preference is due to favorable secondary orbital overlap between the LUMO of the dienophile with the HOMO of the diene. However, this explanation is contested in the literature.[46-48] Of the two molecular orbitals for the dienophile and the four molecular orbitals for the diene, the reaction is considered to be the result of an interaction between the LUMO of the dienophile and the HOMO of the diene (Figure 8 **B** and **C**). The

energy difference (ΔE) between the LUMO of the dienophile and the HOMO of the diene governs the rate of the Diels-Alder reaction. Two simple strategies known to increase the rate of the Diels-Alder reaction (decrease ΔE) are illustrated in part **C** of Figure 8. The LUMO of the dienophile can be lowered in energy by the addition of electron withdrawing groups such as an aldehyde as shown for acrolein. Alternatively, the HOMO of the diene can be raised in energy by the addition of electron donating groups as illustrated by the well-known Danishefsky diene (**D** in Figure 8).[49] In addition to the traditional intermolecular Diels-Alder reaction, the intramolecular version, in which the dienophile is linked to the diene, has been shown to be entropically favored over its intermolecular counterpart. This allows for an accelerated reaction between dienophiles and dienes which would otherwise be considered unreactive.

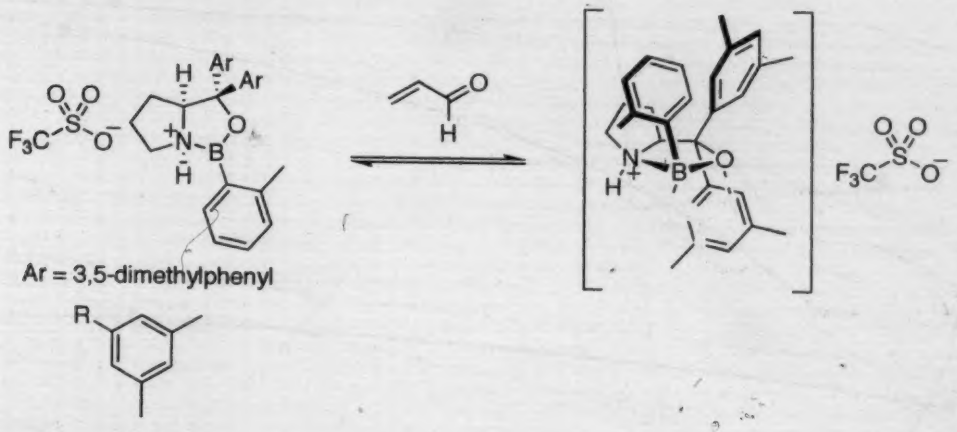
Solvents are well known to affect the rate and selectivity of the Diels-Alder reaction. It was discovered that water can accelerate the Diels-Alder reaction up to 12 800 times over the same reaction performed in an organic solvent.[50, 51] The reactants (dienophile and diene) aggregate in aqueous media as they are relatively hydrophobic and the close proximity is thought to increase the rate of reaction. The *endo* selectivity of the reaction can be increased as the *endo* transition state is more compact (less surface area exposed to water) than the *exo* transition state. In addition to these enforced hydrophobic interactions, the water molecules can form hydrogen bonds with the EWG of the dienophile and

activate the dienophile in the same way as a Lewis acid. The effect of water on the rate and selectivity of the Diels-Alder has been studied extensively.[51]

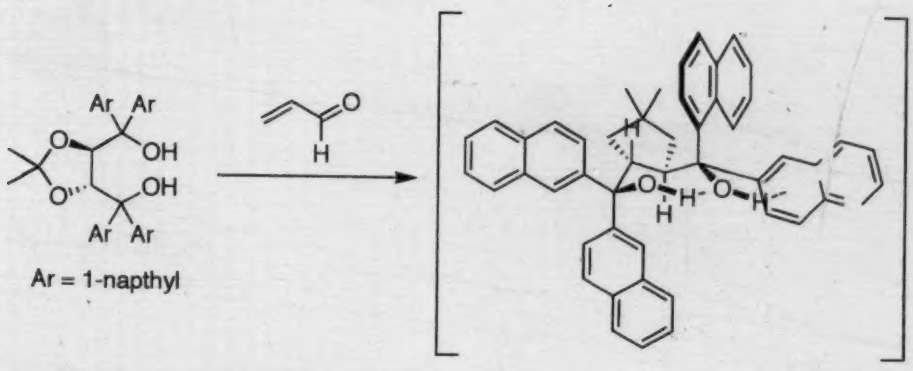
Just as addition of an EWG to the dienophile will increase its rate (lowers the energy of the LUMO of the dienophile) of reaction, so will increasing the electronegativity of the electron withdrawing group.[52] This can be achieved by using three different types of activating agents which are Lewis acids[52, 53], Brønsted acids[54, 55] and organocatalysts.[56, 57] The dienophile typically has a carbonyl attached as this gives an already reactive dienophile but more importantly the lone pairs on the carbonyl oxygen provide a handle to which the catalyst can bind. In all three examples the origin of chiral induction is believed to result from a reversible, strong bond to an electron deficient group and blockage of an enantiotopic face of the dienophile double bond (Scheme 10).

Scheme 10 Selected chiral catalysts used in the Diels-Alder reaction

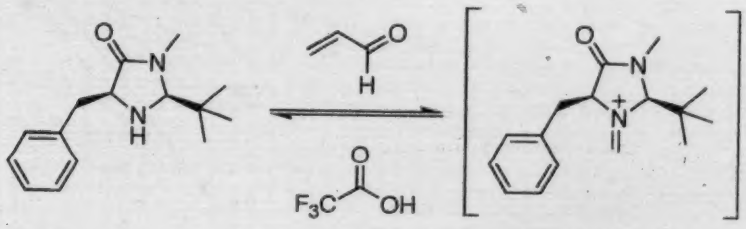
Lewis Acid: Oxazaborolidine



Bronsted Acid: TADDOL



Organocatalysis: Chiral Amine

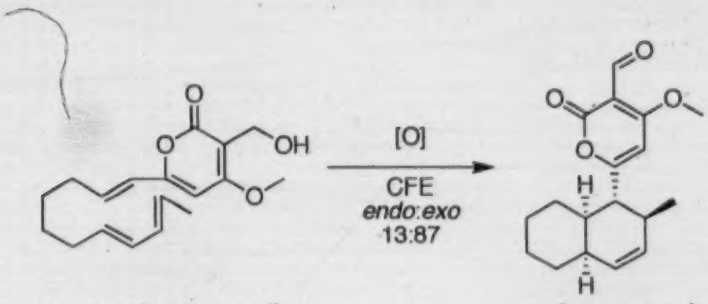


1.4.1 Known Natural Diels-Alderases

A number of Diels-Alderases in biosynthetic pathways appear to occur in fungal polyketides. Examples include the biosyntheses of solanopyrone A (**25**), lovastatin (**1**), galiellalactone (**26**) and macrophomate (**27**) (Scheme 11).[39, 58-61] Isolation of the enzymes responsible for the Diels-Alder activity in these biosynthetic pathways has proven to be difficult. The only two isolated to date are involved in the biosynthesis of **1** and in the biosynthesis of **27**.[39, 62] The Diels-Alderase enzyme responsible for the formation of **27**, macrophomate synthase (MPS), has been studied extensively and is the only Diels-Alderase to date for which an X-ray crystal structure has been obtained.[61, 62]

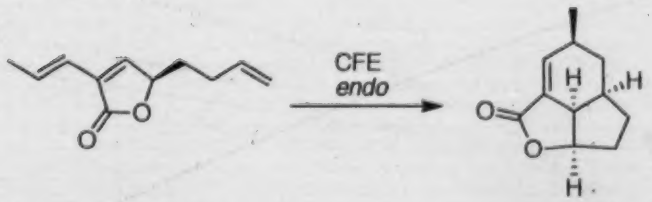
It is thought that Diels-Alderase enzymes induce chirality by encapsulating the substrate in a chiral environment (the active site). This idea is supported by the fact that in the absence of enzyme a reversal of selectivity in the Diels-Alder reaction (*endo* instead of *exo*) is seen for solanopyrones and no product with the correct stereochemistry is formed for the reaction catalyzed by LNKS.[39, 63] The enzymes accelerate the Diels-Alder reaction, as found for (-)-pregaliellalactone presumably by activation of the dienophile in the same manner as a Lewis acid.[59, 60] In the case of solanopyrone biosynthesis, oxidation of a primary alcohol to an aldehyde is utilized as a trigger for the reaction.[63]

Scheme 11 Examples of transformations mediated by a natural Diels-Alderase enzyme



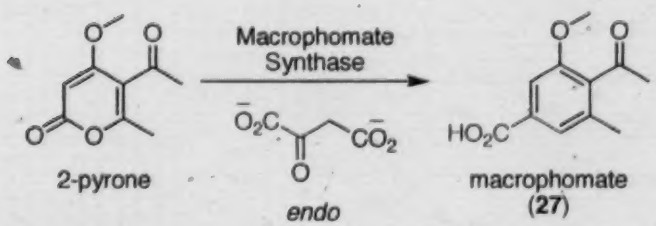
prosolanopyrone II

solanopyrone A
(**25**)



(-)-pregaliellalactone

(+)-desoxy-galiellalactone
(**26**)



2-pyrone

macrophomate
(**27**)

CFE = Cell Free Extract

1.5 The Goals of This Thesis

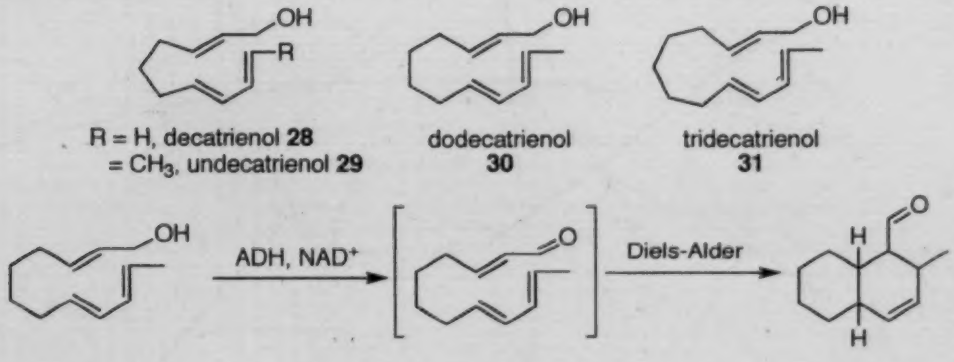
The first goal of this thesis is to gain insight into the various factors which govern enzyme catalyzed Diels-Alder reactions by mimicking this process *in vitro*. To achieve this we chose to explore the possibility of using an alcohol dehydrogenase enzyme to catalyze the Diels-Alder reaction of suitable triene substrates. The cyclized products could then be used to develop a synthesis of analogs of DHL **13** in labeled and unlabeled form. A new lactone precursor could be reacted with the cyclized products to achieve this goal. The post-PKS transformations of **13** remain controversial. It is not known if monacolin L (**21**) and 3α-3,5-hydroxy-DHL (**20**) are true biosynthetic intermediates or are artifacts of extraction.[64] Thus, the second goal of thesis is to develop a synthetic route to these two compounds so that they can be tested to determine whether they are intermediates or shunt metabolites.

CHAPTER 2. Results and Discussion

2.1 Synthesis of Test Substrates for Alcohol Dehydrogenase Oxidation

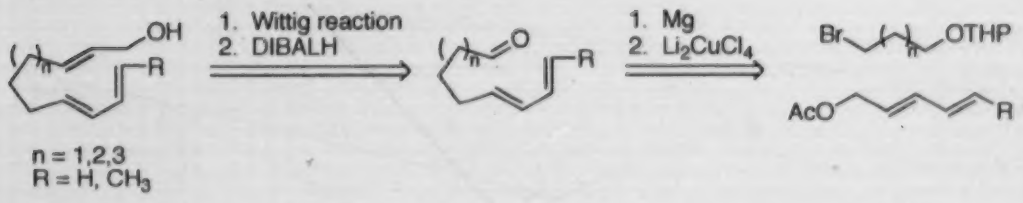
The inspiration to use an alcohol dehydrogenase enzyme (ADH) to catalyze both an oxidation and a Diels-Alder cyclization came from the biosynthesis of solanopyrones. As described in Section 1.4.1, a protein oxidizes a primary alcohol to an aldehyde, which is followed by an *exo* selective Diels-Alder reaction (Scheme 11).[58] The α,β-unsaturated aldehyde is known to be a potent dienophile for the Diels-Alder reaction as was discussed in Section 1.4. The substrates required to apply this concept to ADH reactions are shown in Scheme 12.

Scheme 12 Test substrates and the proposed ADH oxidation induced Diels-Alder reaction



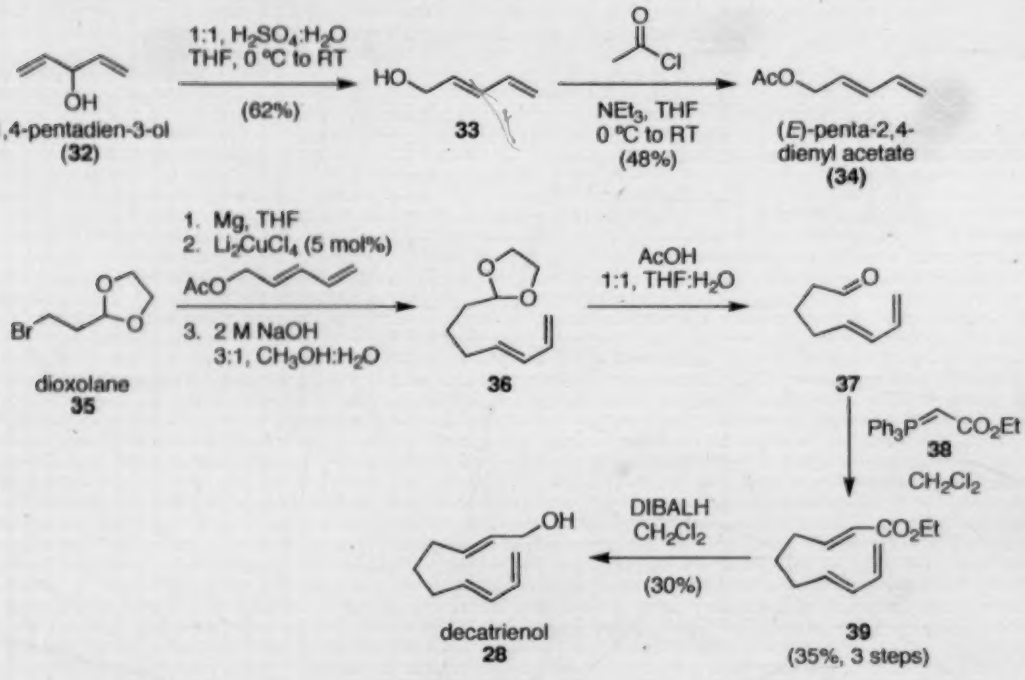
We envisioned synthesizing the test substrates using a cuprate catalyzed alkylation as the key bond forming step.[65, 66] This would install the diene in one step and would maintain the required *trans* geometry of both double bonds. An *E*-selective Wittig olefination followed by reduction would then furnish the requisite triene alcohols (Scheme 13).[67]

Scheme 13 Retrosynthetic analysis of the test substrates



The synthesis of decatrienol **28** requires (*E*)-penta-2,4-dienyl acetate (**34**) which is readily synthesized from 1,4-pentadien-3-ol (**32**) (Scheme 14).[68-70] The pentadienyl acetate **34** contains three percent of the *cis* isomer as determined from the ^{1}H NMR spectrum. Initial attempts to couple 2-(3-bromopropoxy)tetrahydro-2*H*-pyran with pentadienyl acetate **34** met with failure as the Grignard reagent derived from 2-(3-bromopropoxy)tetrahydro-2*H*-pyran is not stable. However, using 2-(2-bromoethyl)-1,3-dioxolane (**35**) works very well in this context and saves a synthetic step. Separation of unreacted pentadienyl acetate **34** from dioxolane octadiene **36** is very difficult. Basic hydrolysis of the acetate to form diene alcohol **33** effectively resolves the purification problems.

Scheme 14 Synthesis of decatrienol **28** and (*E*)-penta-2,4-dienyl acetate (**34**)

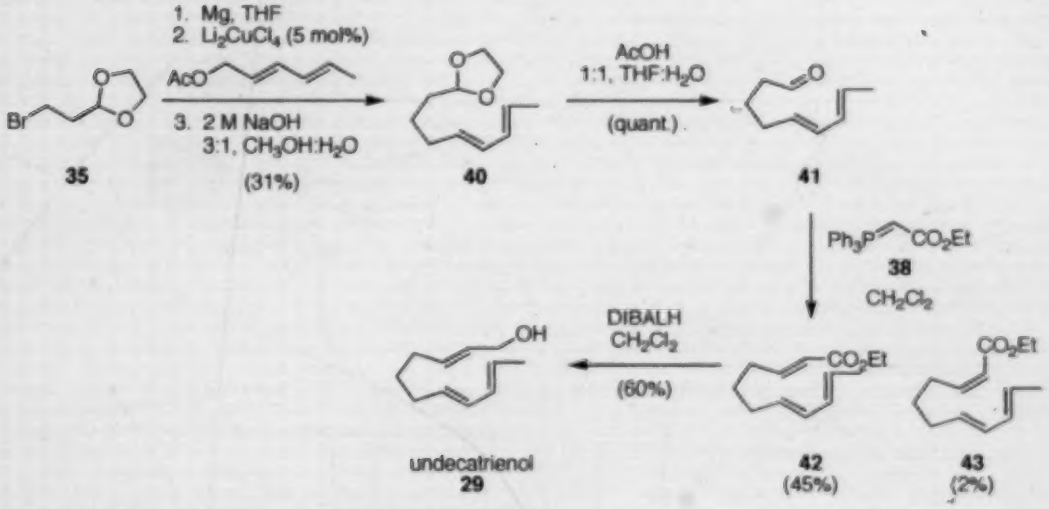


The product resulting from γ-substitution of the hexadienyl acetate to **35** could not be separated from the desired α-substituted product **40**. Therefore the mixture of products were carried through to the decatrienoate **39** at which point separation was possible. The preparation of the stabilized Wittig reagent (**38**) is trivial, and it is prepared on a multi-gram scale and stored at room temperature without any complications.[71] The Wittig olefination provides high *trans* selectivity (9:1, *trans*:*cis* according to ^{1}H NMR) and the *cis* isomer is readily separated from the *trans* isomer using flash chromatography. In addition, the Wittig reaction is much

more rapid in dichloromethane as compared to toluene (3 hours versus 16 hours).

The synthesis of undecatrienol **29** (Scheme 15) is identical to the synthesis of decatrienol **28** except a dienyl acetate with a terminal methyl group is required (Scheme 15).

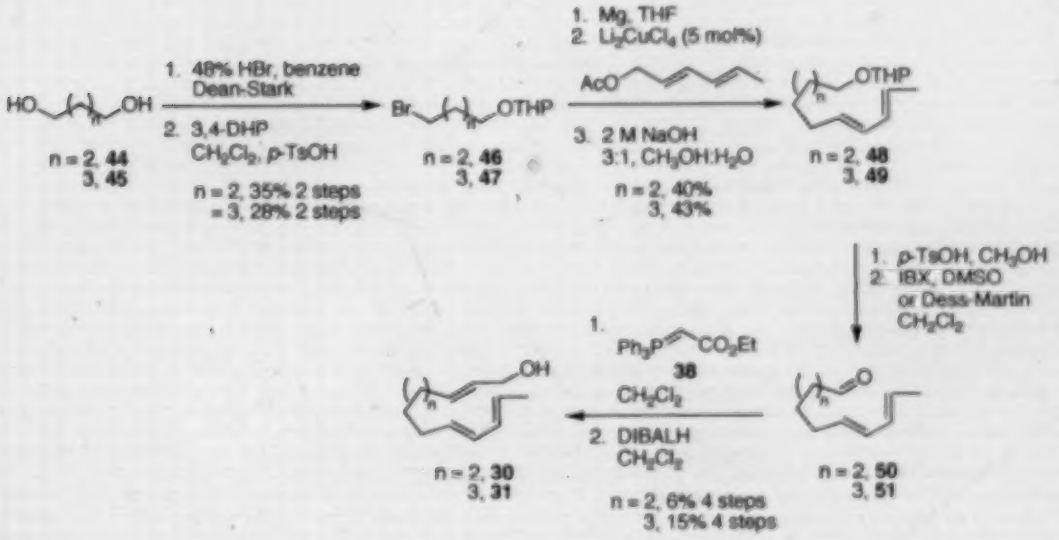
Scheme 15 Synthesis of undecatrienol **29**



For the synthesis of undecatrienol **29** the side product from the cuprate reaction is readily removed using flash chromatography with silver nitrate impregnated silica gel.

The remaining two substrates, dodecatrienol **30** and tridecatrienol **31** were synthesized in a very similar manner (Scheme 16).

Scheme 16 Synthesis of dodecatrienol **30** and tridecatrienol **31**

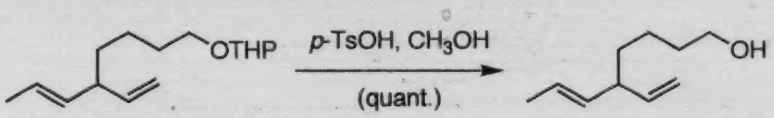


The first transformation of the synthesis, monobromination of the symmetric diols **44** and **45** works very well on large scale (Scheme 16).[72] Protection of the primary alcohol as the THP ether provides compounds **46** and **47** and proceeds without any complications. However, the cuprate catalyzed reaction is particularly troublesome in these syntheses because of the relatively low yields and difficulties in the purification of the THP diene products (**48** and **49**). Oxidation of the diene alcohols **48** and **49** by Dess-Martin Periodinane[73-75] is sluggish and affords low yields of diene aldehydes **50** and **51** (Scheme 16).

However, *o*-iodoxybenzoic acid (IBX) is a far superior choice of oxidant in these syntheses.[75, 76] Preparation of IBX[77] is also more reliable than the preparation of the Dess-Martin reagent.[74] Reduction of the triene ethyl esters in all syntheses using DIBALH minimized the amount of conjugate reduction that occurred in the last step. However, there was usually a small amount of this by-product present. This side product was very difficult to remove but was not expected to interfere with the ADH reaction, so therefore the crude triene alcohols were used directly in the reactions and were not fully characterized.

The cuprate catalyzed reaction of the Grignard reagents to the dienyl acetates suffers from competing γ-substitution. To confirm this is indeed the case, the γ-substituted product **52** was isolated from the preparation of 2-((6*E*,8*E*)-deca-6,8-dienyloxy)tetrahydro-2*H*-pyran (**48**). The analogous γ-substituted products are formed in varying yields for all of the syntheses, but in this case it was isolated in a yield of 7 percent. Compound **52** is isolated as a mixture of two diastereomers after flash chromatography employing silver nitrate impregnated silica gel (Scheme 17). To simplify the spectral data, the THP group is removed to provide the corresponding alcohol **53** (Scheme 17).

Scheme 17 Structure of the major side product **52** from the cuprate catalyzed reaction and conversion to the corresponding alcohol **53**



52 **53**

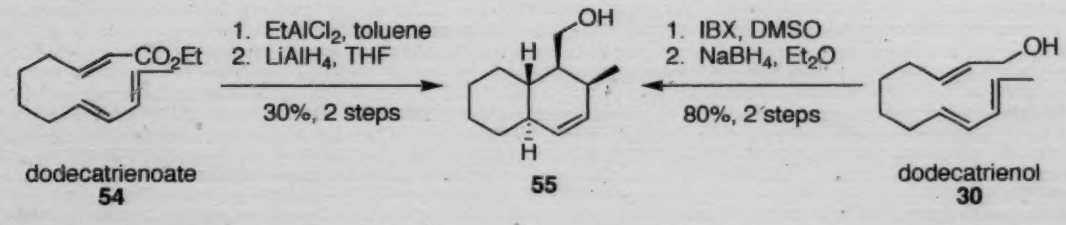
This problem is not mentioned in some early papers[65, 66] that report the use of this reaction. However, this was noticed by Roush and Gillis in their study of intramolecular Diels-Alder reactions.[78] This type of cuprate catalyzed reaction is well-known in the literature.[79-81] In particular, the use of dilithium tetrachlorocuprate (Li_2CuCl_4) was found to be sensitive to reaction conditions and control over the outcome (high α to γ ratio) could be obtained.[79] There are two ways to remove the undesired side product; proceed to the triene ethyl ester (as in the synthesis of **39**, Scheme 14) without purification or by purification with flash chromatography using silver nitrate impregnated silica gel.

2.2 Chemical Cyclization and Analysis

In order to be able to assess the diastereoselectivity and enantioselectivity of the cyclization reactions in the presence of the ADH enzymes, chiral GC-MS was used (Chapter 3.3). This method was applied previously in our group for the analysis of the cyclization of ethyl dodecatrienoate.[67] In order to have a more convenient method of analysis, we decided to analyze the products of the

reactions as the alcohols rather than the corresponding esters (Scheme 18). To this end, decalin alcohol **55** was synthesized in two different ways as shown in Scheme 18.

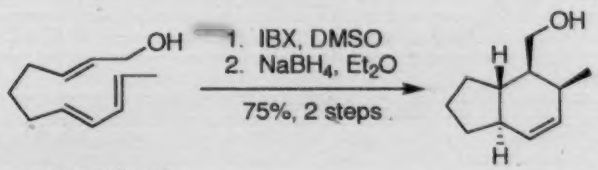
Scheme 18 Preparation of the standard decalin alcohol **55** for GC-MS analysis



dodecatrienoate **54** — **55** — dodecatrienol **30**

Based on extensive literature precedent it is known that Lewis acid catalysis provides high *endo* diastereoselectivity.[78] Therefore, ethylaluminum dichloride was utilized followed by reduction to provide racemic decalin alcohol **55** (Scheme 18).[67] In order to test conditions that would be used for the enzyme reactions, IBX oxidation followed by reduction with sodium borohydride provides the same racemic decalin alcohol **55** in a higher yield over two steps. This method was expected to provide the four stereoisomers expected for an uncatalyzed Diels-Alder reaction. However, cyclization under the IBX oxidation conditions provided the *endo* products with very high diastereoselectivity. This suggests that some species in the oxidation reaction is acting as a catalyst for the Diels-Alder reaction. This outcome was the same for the cyclization of undecatrienol **29** to indene alcohol **56** (Scheme 19).

Scheme 19 Chemical oxidation and cyclization of undecatrienol **29**



undecatrienol
29

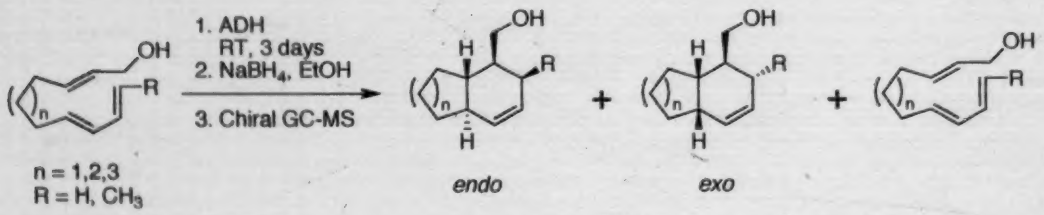
56

Analysis of the decalin alcohol **55** by GC-MS using a chiral column allowed the assignment of the *exo* products (62.5 and 63.2 minutes) and the *endo* products (64.9 and 65.7 minutes). This was the model substrate used to probe the outcome of the ADH catalyzed reactions.

2.3 Alcohol Dehydrogenase Oxidations

Three commercially available ADH enzymes were tested for their ability to oxidize and cyclize the test substrates. The enzymes tested were yeast alcohol dehydrogenase (YADH), horse liver alcohol dehydrogenase (HLADH) and hydroxysteroid dehydrogenase (SDH) from *Pseudomonas testosteroni* (ATCC 11996).

Scheme 20 Summary of the results of the ADH catalyzed reactions



n = 1,2,3
R = H, CH_3

For these reactions the substrate is either added neat or as a solution in acetone to the enzyme reaction. Acetone is tolerated by the enzymes and it is capable of regenerating the cofactor to its oxidized form via reduction of the carbonyl of acetone by the ADH enzymes.[82, 83] In all of the conditions tested the cyclized products as well as the starting triene were isolated (Scheme 20).

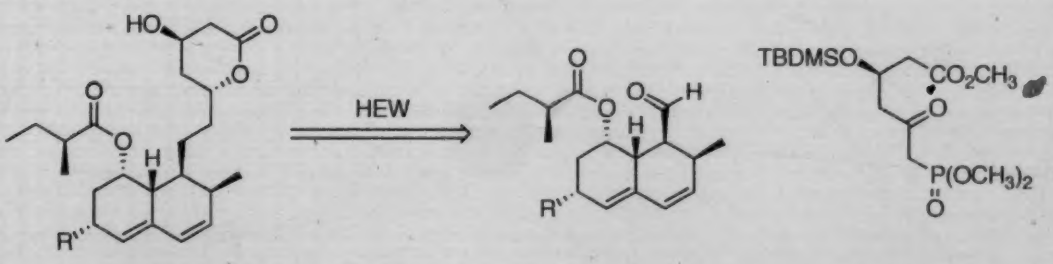
The Diels-Alder reaction in the presence of the ADH enzymes is not selective. The lack of enantio or diasteroselectivity of the ADH catalyzed reactions indicates that the desired Diels-Alder reaction takes place in the solvent rather than in the chiral environment of the active site of the enzyme. The long reaction times (three to five days) as well as the low yields (ca. 2%) also suggests that these substrates may be too large to fit in the active site. This result is supported by the very slow (5 days) and low yield (< 5% yield) of oxidation of **55** by HLADH. The reaction rate and yield would be expected to be greater if the decalin alcohol **55** could fit in the active site.

Even though the ADH catalyzed reactions did not work as expected, the decalin alcohol **55** could be used to synthesize desmethyl analogs of DHL **13**. To achieve that goal, a suitable lactone precursor needed to be synthesized.

2.4 Construction of the Lactone Precursor

A typical approach to the total synthesis of lovastatin (**1**) and compactin (**3**) is to use Masamune's[84] variant of the Horner-Emmons-Wadsworth (HEW) coupling of a lactone fragment (ester phosphonate **58**) to a decalin aldehyde **57** (Scheme 21).[85, 86]

Scheme 21 Retrosynthetic analysis of lovastatin (**1**) and compactin (**3**) which utilizes a HEW reaction as the key bond forming step



R = CH_3, lovastatin (**1**)
H, compactin (**3**)

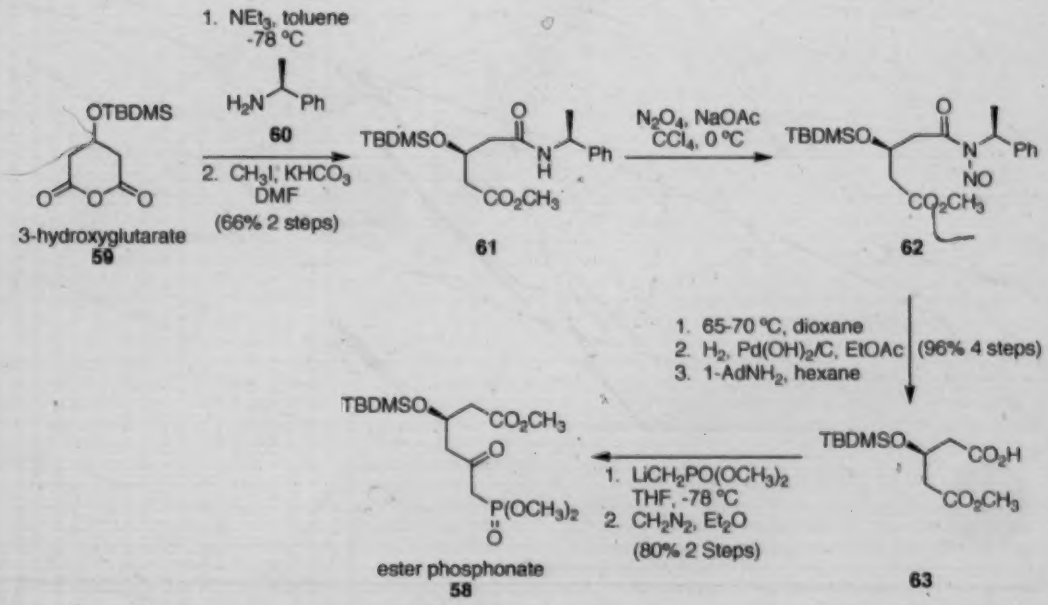
decalin aldehyde
57

ester phosphonate
58

There are numerous syntheses reported for the preparation of ester phosphonate **58**.[87-89] We chose to modify a route published by researchers at Bristol-Myers Squibb.[90] One of the difficulties encountered in the syntheses of protected 3-

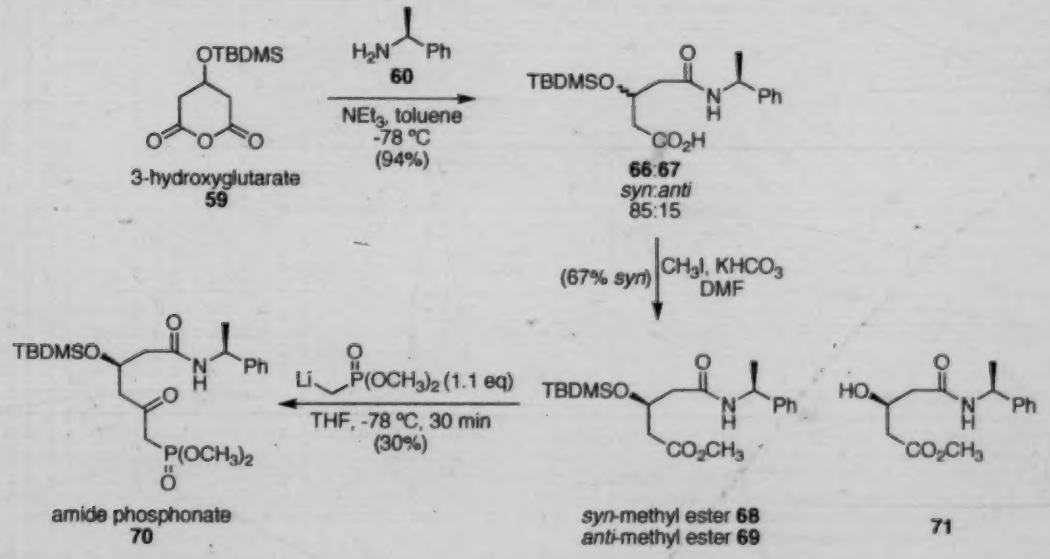
hydroxyglutarate chiral synthons such as **58**, is β-elimination of the OTBDMS group under the strongly basic conditions required to introduce the phosphonate. To shut down the β-elimination pathway, the BMS researchers used deprotonation of the acid in **63** (Scheme 22).[90] This strategy increases the pKa of the hydrogen atoms alpha to the carboxylate and thus hinders the elimination reaction. This strategy provided the ester phosphonate **58** in 80% yield with no competing β-elimination. However, while successful, this route has more steps than previous syntheses. The reason is due, in part, to the necessity of selective hydrolysis of an amide in the presence of a more reactive ester. To achieve this goal, the researchers utilized the Huisgen-White rearrangement of the nitroso-amide **62** to yield the requisite half ester intermediate **63** after hydrogenolysis with Pearlman's catalyst.[90, 91]

Scheme 22 Synthesis of ester phosphonate **58** by researchers at Bristol-Myers Squibb



It seemed possible to install the phosphonate group directly from the ester amide intermediate **61**. The amide proton would be expected to react with the first equivalent of lithium phosphonate. This would serve the same purpose as the carboxylate anion in the original synthesis and prevent β-elimination. Furthermore, the synthesis would be three steps instead of eight and it would obviate the need for toxic N_2O_4 and diazomethane.

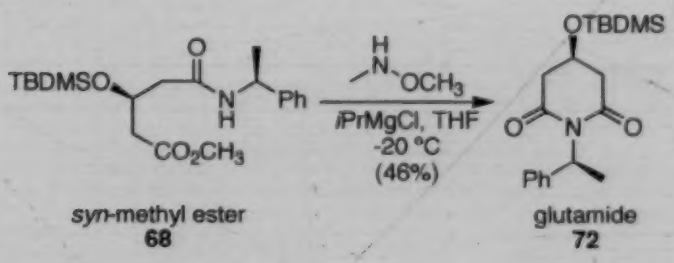
Scheme 23 Synthesis of a new lactone precursor, amide phosphonate **70**



The modified synthesis commences in the same fashion as that published previously (Scheme 23). The commercially available anhydride **59** reacts with (*S*)-(-)-α-methylbenzylamine (**60**) to diastereoselectively provide amide acids **66** and **67**, in high yield. Separation of the diastereomers at this stage is very tedious. It is much easier to separate *syn* from *anti* diastereomers after conversion of the mixture of acids (**66** and **67**) to the corresponding methyl esters. Flash chromatography then provides a 67% yield of the desired *syn*-methyl ester **68**. The next reaction, condensation with the lithium phosphonate anion, is very challenging. The best yields of amide phosphonate **70** without competing β-elimination are obtained when 1.1 equivalents of lithium phosphonate anion are used with respect to the *syn*-methyl ester **68**. The anion

needs to be added over 25 minutes and the reaction must be performed using 100-150 mg of **68**. Both increasing the amount of **68** and the rate of addition increased the amount of β-elimination (usually in the range of 10% elimination). Increasing the reaction time also increases the amount of β-elimination. It therefore appeared that deprotonation of the amide did not provide any beneficial effect on the selectivity of the reaction. However, what is sacrificed in overall yield (18% versus 50% published yield) is compensated by a shorter synthetic route. Upon storage of *syn*-methyl ester **68** the silyl group is lost to provide the free alcohol **71** (Scheme 23). However, the presence of the free alcohol **71** did not interfere with the formation of amide phosphonate **70**. In an effort to improve the yield of the final step, the methyl ester was activated by converting it into a Weinreb amide (Scheme 24). This strategy was employed previously by Heathcock and co-workers to improve their synthesis of a lactone precursor.[89]

Scheme 24 Attempted conversion of the *syn*-methyl ester **68** into the corresponding Weinreb amide



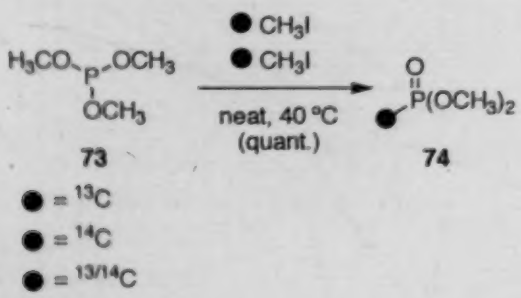
syn-methyl ester **68**

glutamide **72**

As the silyl protecting group is sensitive to acidic conditions,[92] the introduction of the Weinreb amide is conducted under the basic conditions developed by researchers at Merck.[93] The desired reaction did not occur, but rather the chiral glutamide **72** shown in Scheme 24 is isolated in 46% yield. This result indicates that the Weinreb amide is formed, but under the basic conditions the amide is trapped with the internal nucleophile formed by deprotonation of the pre-existing amide. This type of reactivity had not been observed for *syn*-methyl ester **68** when it was reacted with the lithium phosphonate anion despite the fact that the amide is certainly deprotonated in this process.

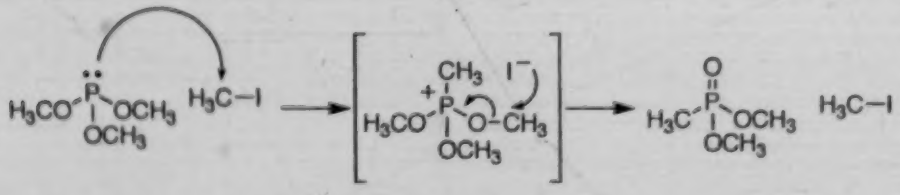
In devising the synthesis outlined in Scheme 23, we were cognizant of the fact that we would need to be able to construct the amide phosphonate **70** with carbon-13 and carbon-14 labels. This is required for biosynthetic studies of DHL (**13**) analogs as well as monacolin N (**76**). The isotopic labels could be incorporated into the lactone by the synthesis of labeled dimethyl methylphosphonate (**74**) using the Arbuzov reaction (Scheme 25).[94]

Scheme 25 Arbuzov reaction to produce labeled dimethyl methylphosphonate (**74**)



This reaction proceeds with quantitative yield and isotopic incorporation. The high isotopic incorporation is in accord with the published procedure[94] and can be explained by the accepted mechanism for the Arbuzov reaction (Scheme 26).

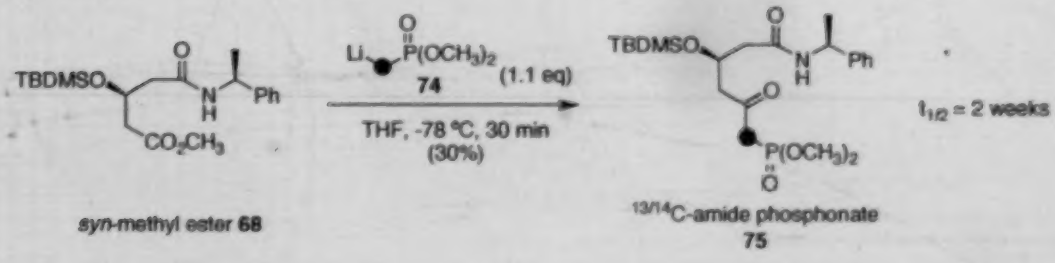
Scheme 26 Mechanism of the Arbuzov reaction



The reaction has been shown to proceed in two steps where the second step is rate limiting.[94, 95] Thus, all of the methyl iodide (labeled in this case) reacts to form the charged intermediate and there is no competition from the unlabeled methyl iodide which is released in the second step. The labeled dimethyl

methylphosphonate **74** is then used to prepare the $^{13/14}$C-amide phosphonate **75** (Scheme 27). Compound **75** is prepared in comparable yield to amide phosphonate **70** but is unfortunately very unstable. This was surprising as the unlabeled version was found to be completely stable even when stored for many months. Furthermore, the reaction time for the HEW coupling reaction can be as long as the half life of **75** thus rendering this reagent unsuitable for the preparation of labeled analogs of DHL **13** or monacolin N (**76**).

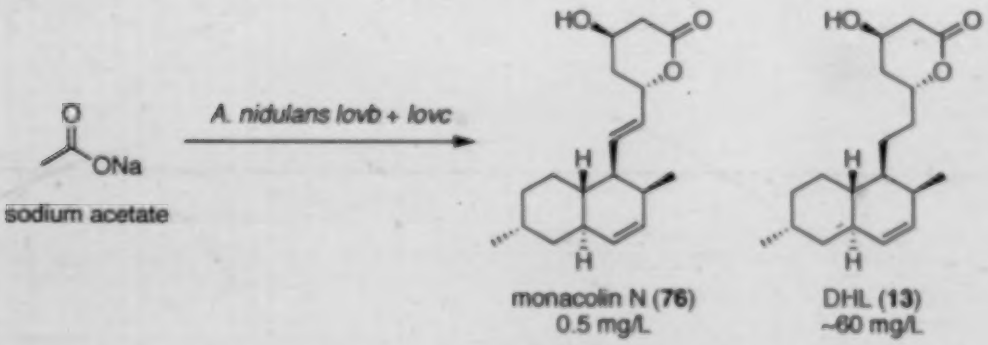
Scheme 27 Preparation of $^{13/14}$C-amide phosphonate **75**



2.5 Attempted Elaboration to DHL 13 Analogs and Monacolin N (76)

Our laboratory recently discovered a new metabolite in the fermentation cultures of *A. nidulans lovb + lovc*.[96] During isolation of large amounts of DHL **13**, monacolin N (**76**) was isolated and characterized from the less polar fractions of the crude extract (Scheme 28).[96]

Scheme 28 Isolation of the new metabolite, monacolin N (**76**), from *A. nidulans lovb + lovc*

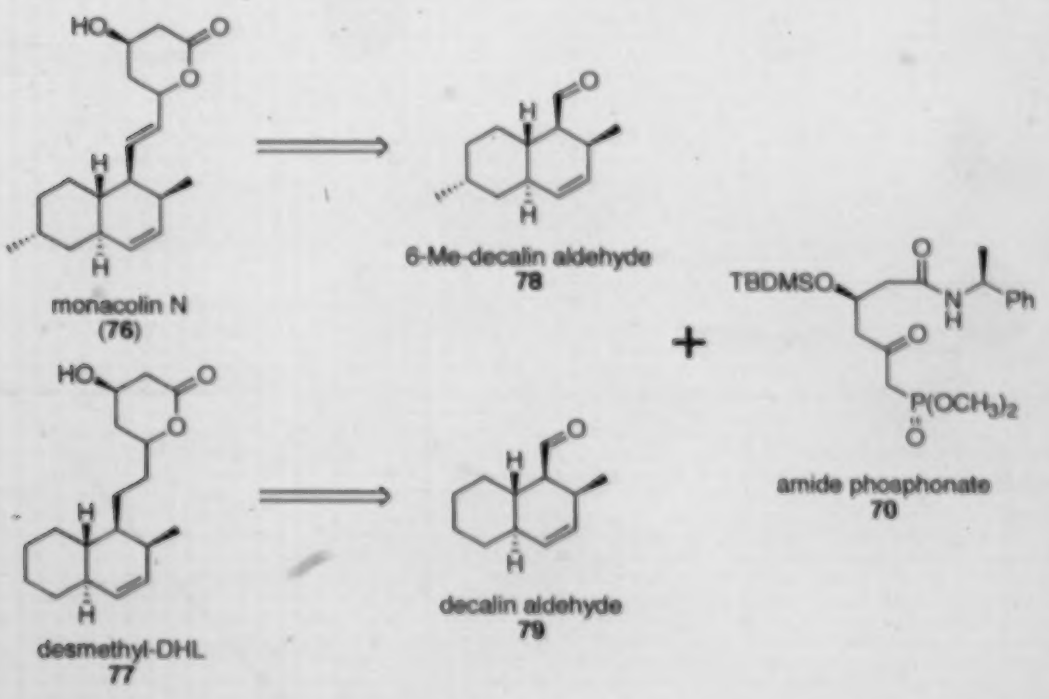


It is produced at an approximate level of 0.5 mg/L. It is interesting to speculate about the origin of the double bond between the lactone and dihydronapthalene ring. It appears the ER activity fails at the level of the heptaketide and that this error is tolerated by the LovB protein.[96]

As the lactone precursor (amide phosphonate **70**) was available to synthesize desmethyl-DHL analog **77**, it was decided that the total synthesis of monacolin N

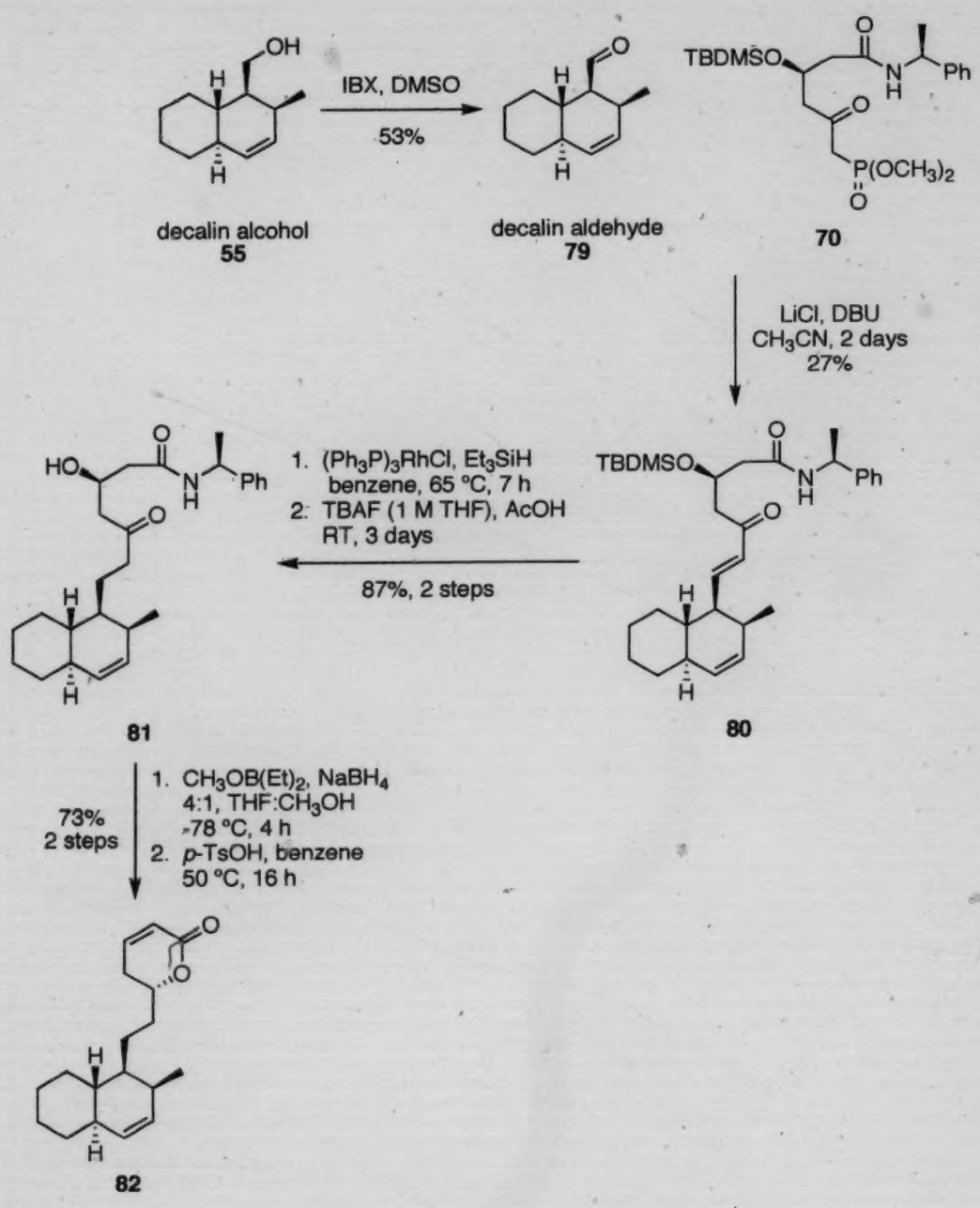
(**76**) could be be pursued as well. Both targets, monacolin N (**76**) and desmethyl-DHL **77** could be used to probe the substrate selectivity of the post-PKS enzymes (Scheme 29).

Scheme 29 Retrosynthesis of monacolin N (**76**) and desmethyl-DHL **77**



With the required amidephosphonate **70** in hand and the two decalin aldehydes, **78** and **79**, the syntheses of monacolin N (**76**) and desmethyl-DHL **77** were pursued following the literature route for attachment of the upper half (lactone precursor).[65, 66, 68]

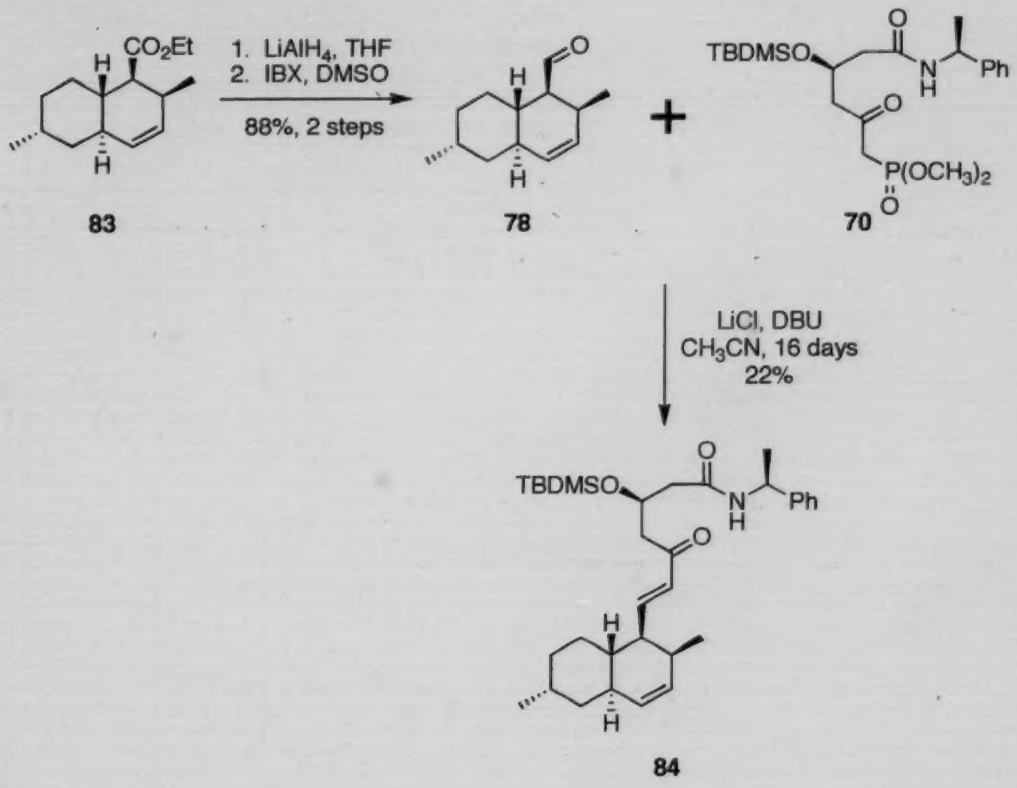
Scheme 30 Attempted synthesis of desmethyl-DHL **77**



The synthesis starts with the oxidation of decalin alcohol **55** to decalin aldehyde **79** by IBX. This reaction is high yielding but the product is volatile which results in a low isolated yield. The next reaction, Masamune's variant of the HEW reaction, was found to be unreliable.[84, 88] The yield ranged from 8% to 27% even in reactions which were performed in parallel under identical conditions. The primary problems were elimination of the OTBDMS group and epimerization of the unreacted aldehyde (22% by ^{1}H NMR). These results are in accord with literature reports, although the yields obtained with an ester present, rather than a secondary amide, were much higher.[86, 89] The α,β-unsaturated ketone **80** was subjected to conjugate reduction by Wilkinson's catalyst[88, 97] followed directly by cleavage of the silyl protecting group using TBAF under buffered conditions with acetic acid.[98] The intermediate could not be isolated and characterized as there was aggregation of the product with a silyl species. Breaking this aggregate requires long reaction times with TBAF/AcOH (3 days, Scheme 30). The following chelation-controlled reduction utilizing conditions developed by researchers at Sandoz[99] proceeds smoothly to afford the *syn*-1,3-diol. The initial product of the reaction is a boron chelate with the *syn*-1,3-diol that could be cleaved by heating in methanol.[88] The diol product is then subjected to lactonization conditions which provides the unexpected elimination product **82** as shown in Scheme 30.[100]

The synthesis of monacolin N (**76**) begins with reduction of the ethyl ester **83** (**83** was generously provided by Dr. Doug Burr) with subsequent oxidation by IBX to 6-Me-decalin aldehyde **78** which proceeds with a high yield over two steps. Since the aldehyde is volatile it is used directly in the next reaction. The HEW reaction between 6-Me-decalin aldehyde **78** and amide phosphonate **70** in this case is extremely sluggish and did not provide enough α,β-unsaturated ketone **84** to complete the synthesis of monacolin N (**76**).

Scheme 31 Progress toward total synthesis of monacolin N (**76**)



83 **78** **70**

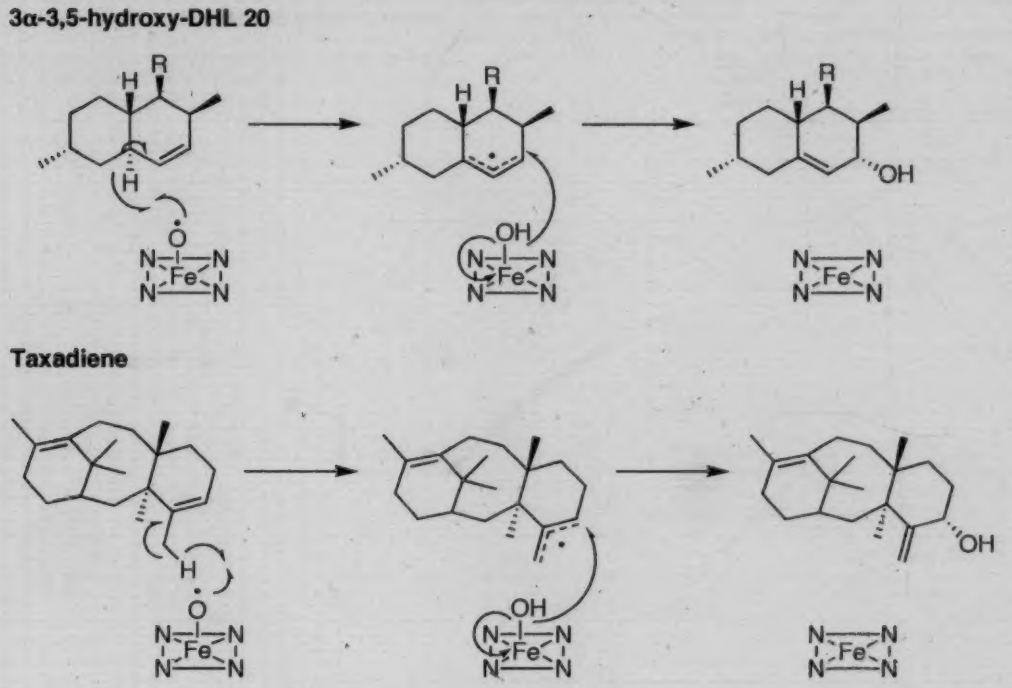
84

Given the results of the last step in the synthesis shown in Scheme 30 and the instability of the $^{13/14}$C-amide phosphonate **75**, the synthesis of monacolin N (**76**) was halted after the HEW reaction (Scheme 31).

2.6 Studies Toward the Synthesis of Monacolin L (21) and 3α-3,5-Hydroxy-DHL (20)

It has been established that DHL **13** and monacolin J (**22**) are converted into lovastatin (**1**) (Scheme 7).[39, 41] However, it remains to be proven that either monacolin L (**21**) or 3α-3,5-hydroxy-DHL **20** are converted into lovastatin (**1**).[101] It is known that after isolation 3α-3,5-hydroxy-DHL **20** will form monacolin L (**21**) by dehydration in acidic conditions.[64, 102] This suggests that the conversion of **20** into **21** is not necessarily enzyme catalyzed. Both **20** and **21** were isolated from cultures of *Aspergillis terreus*[4] and *Monascus ruber*.[3] However, researchers at Merck could not detect monacolin L (**21**) in growing cultures of *A. terreus*. It was only detected after isolation of lovastatin (**1**).[64] This lead to the conclusion that monacolin L (**21**) is an artifact of extraction rather than a biosynthetic intermediate. If 3α-3,5-hydroxy-DHL **20** is a true biosynthetic intermediate, a possible mechanism for its formation is shown in Scheme 32 which is based on literature precedent.[103]

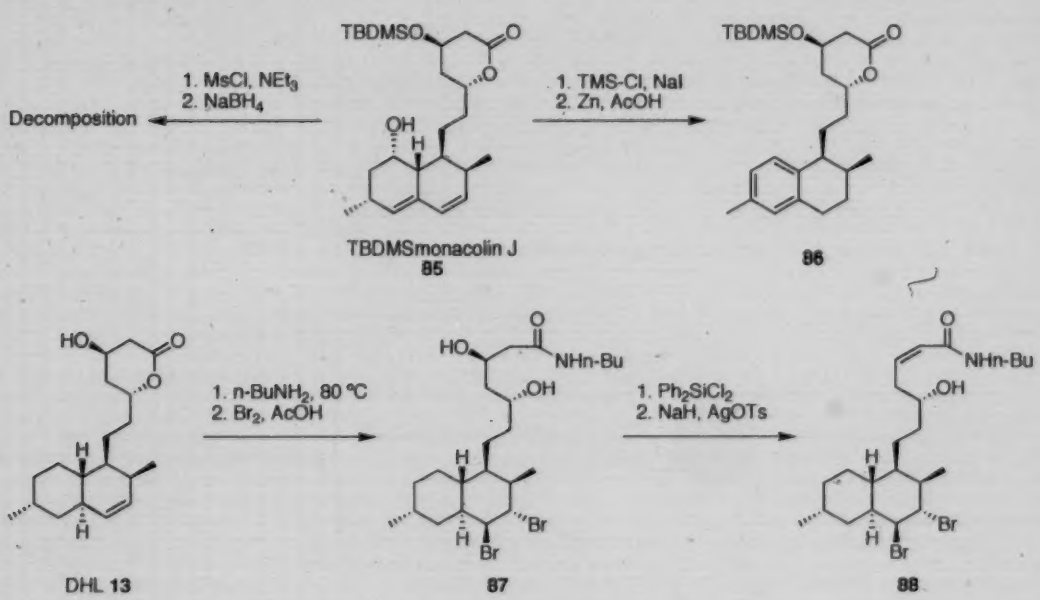
Scheme 32 Proposed mechanism for the formation of 3α-3,5-hydroxy-DHL **20** catalyzed by a cytochrome P450 enzyme similar to a proposed mechanism for the first oxygenation step in the biosynthesis of Taxol[103]



Since it is unclear whether **20** and **21** are biosynthetic precursors, then the order of oxidations proposed for the elaboration of DHL **13** remains in question. Therefore, synthetic routes to **20** and **21** are required in order to test the current hypothesis. To date monacolin L (**21**) and 3α-3,5-hydroxy-DHL **20** are available only by isolation from fermentations of *A. terreus* or *M. ruber*. From a theoretical standpoint there are two approaches to these intermediates, degradation of lovastatin (**1**) or another advanced intermediate and semi-synthesis from DHL **13**. Both approaches were attempted previously by our laboratory, the results are

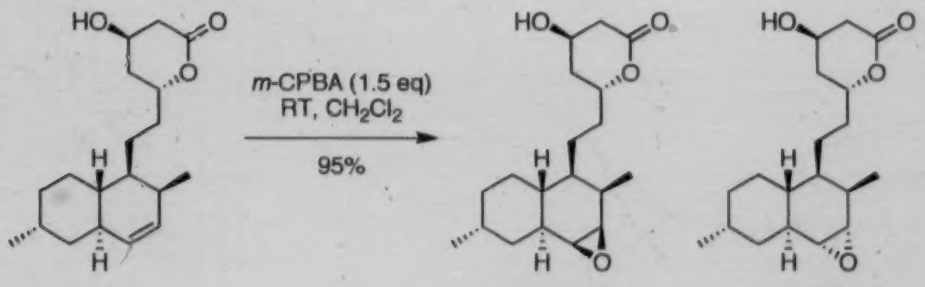
summarized in Scheme 33. The degradation of TBDMSmonacolin J **85** was thwarted as the hydroxyl group at C8 of TBDMSmonacolin J **85** was surprisingly resistant to deoxygenation (Scheme 33).[100] The method which removed the oxygen at C8, iodination followed by reduction with zinc in acetic acid, provided the unexpected aromatized product **86**. Semi-synthesis from DHL **13**, was also attempted. However, the key step, elimination of the dibromide of protected **87** with sodium hydride to form the diene did, not take place as expected (Scheme 33). Despite literature precedent the hydroxyl at C3 was removed and the dibromide remained intact to provide **88** (Scheme 33).[100]

Scheme 33 Attempted syntheses of monacolin L (**21**) from TBDMSmonacolin J **85** and from DHL **13**



Hence, another approach to monacolin L (**21**) was devised for the present study. We decided to start from the epoxide of DHL **13** and explore methods of opening the oxirane ring.

Scheme 34 Epoxidation of DHL **13**



DHL **13**

2 : 1

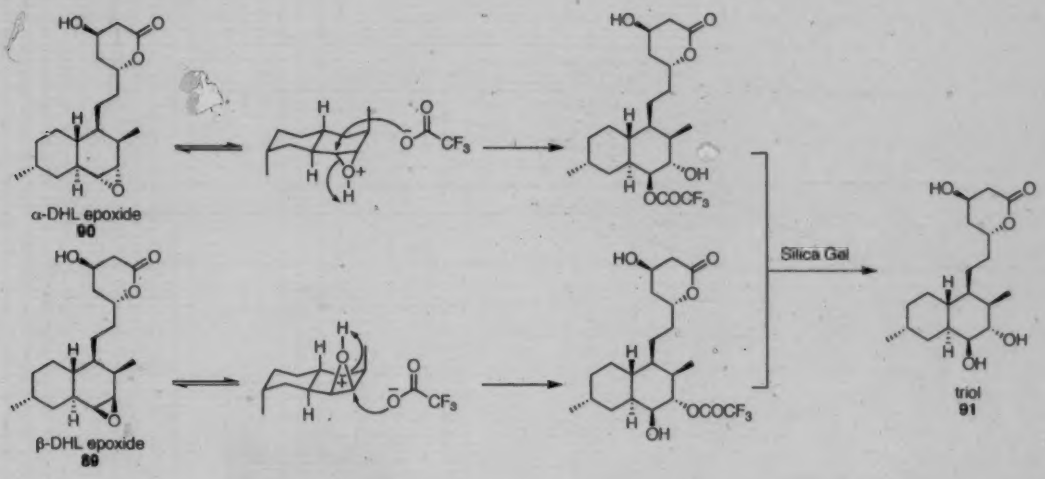
β-DHL epoxide **89**

α-DHL epoxide **90**

The DHL epoxides **89** and **90** are synthesized by reacting DHL **13** with *meta*-chloroperoxybenzoic acid (Scheme 34). It is surprising that the ratio of α to β is 2:1 in favor of β-DHL epoxide **89** (Scheme 34). A possible explanation for this selectivity is a directing effect from the 3-hydroxy group in the lactone ring of DHL **13**. In the present case, epoxidation of the more sterically demanding face of the double bond predominates. With the epoxides **89** and **90** in hand, various reagents were used to test the oxirane ring opening. The reactions were conducted in an NMR tube using CD_2Cl_2 as a solvent and the mixture of β and α-DHL epoxides **89** and **90**. Using flash chromatography, only the α-DHL epoxide **90** is isolated in pure form. Four different reagents, boron trifluoride, lanthanum

triflate, triethylamine and trifluoroacetic acid were tested. Only trifluoroacetic acid reacted with the epoxides and was explored further. The reaction was monitored by TLC, which showed two new spots appearing during the course of the reaction. After 3 days at room temperature a small amount of the starting material remained and the new spots were much more intense than at the start of the reaction. The reaction mixture was then concentrated *in vacuo* and purified by column chromatography. Isolation of two compounds was expected based on the TLC data, however, only one compound eluted from the column. The isolated compound was identified as triol **91** in a yield of 58% (Scheme 35).

Scheme 35 Proposed mechanism for the formation of triol **91** from DHL epoxides **89** and **90**



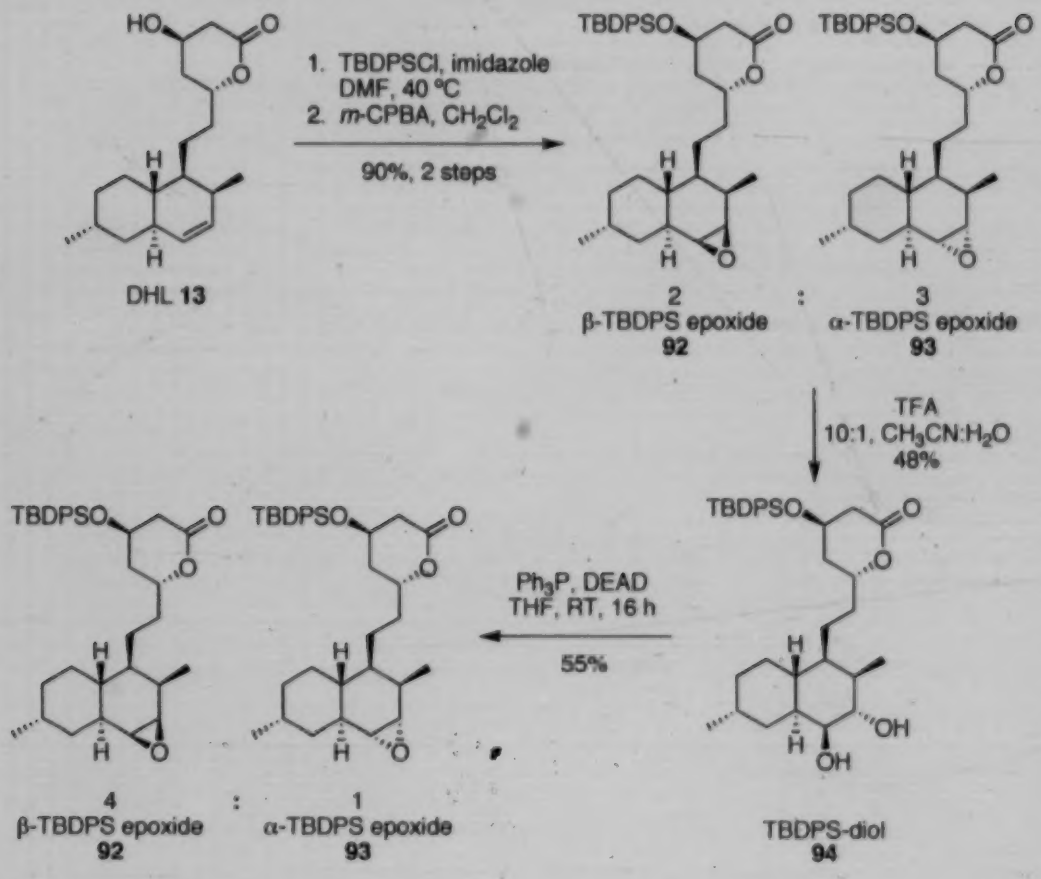
To explain the formation of triol **91** from two different compounds after purification a putative mechanism is proposed (Scheme 35). Once the oxygen of the

epoxide ring is protonated, there are two choices for attack by the trifluoroacetate anion (Scheme 35). This is dictated by the stereochemistry of the epoxide as such species are known to form 1,2-diaxial products as a result of nucleophilic substitution reactions.[104, 105] Once the two adducts are formed, hydrolysis seems to occur on silica gel to form the triol **91**. The hydrolysis of the trifluoroacetate adduct would be expected to be quite facile due to the high electronegativity of the carbonyl carbon. This reaction was also tested using acetic acid. As before, two products were observed on the TLC plate. However, when the reaction mixture was submitted to purification by flash chromatography using silica gel, only starting material was isolated. This suggests that rather than hydrolysis to the diol, the acetate adduct reverts to the epoxide as hydrolysis is not as favorable.

It seemed that this chemistry could be utilized to our advantage. If the alcohol at position 4 could be transformed into a good leaving group, the stereochemistry of the hydrogen at position 4a is of the correct configuration to promote elimination. This would potentially yield the ring system of the 3α-3,5-hydroxy-DHL **20** intermediate or monacolin L (**21**). To accomplish this task the lactone hydroxyl group needed to be protected with a group that is stable to acidic conditions. The *tert*-butyldiphenylsilyl group seemed to be a good candidate.[92] To achieve elimination, dehydration under Mitsunobu reaction conditions could be tested (Scheme 36).[106, 107] The route starts with reaction of the free hydroxyl of DHL **13**

with *tert*-butyldiphenylsilyl chloride followed by epoxidation with *meta*-chloroperoxybenzoic acid.[100]

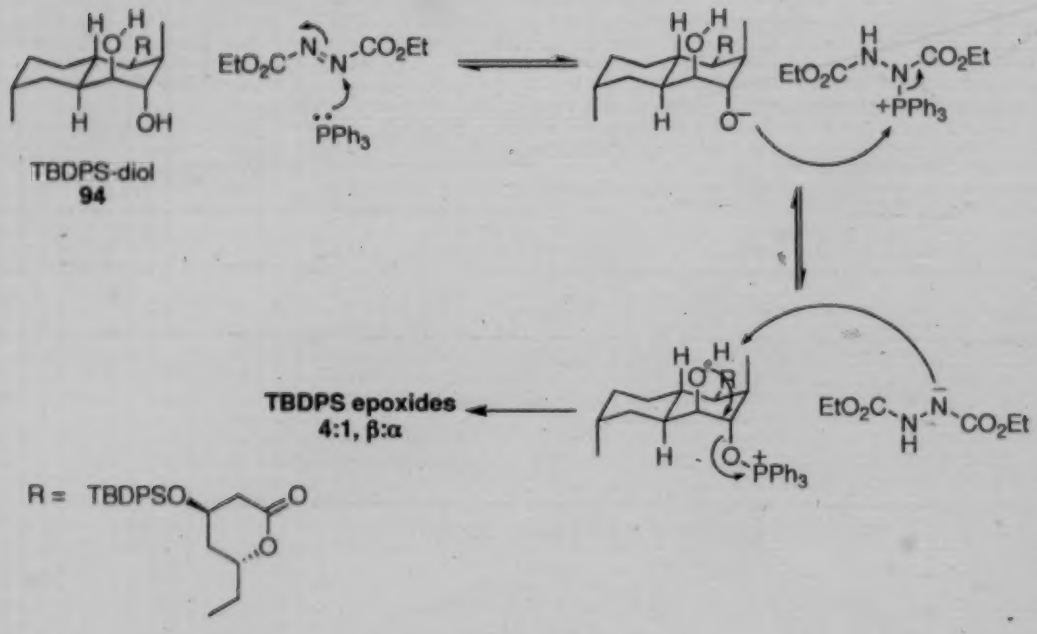
Scheme 36 Results of the Mitsunobu dehydration reaction



This provides the protected epoxides, **92** and **93**, as a 2:3 mixture of β:α (Scheme 36). This is the opposite selectivity to the epoxidation of DHL **13** directly and seems to support the proposal that the 3-hydroxyl has a directing effect (Scheme 34). The reaction of the mixture of epoxides with TFA results in

the formation of TBDPS diol **94** in a yield of 48%. The TBDPS diol **94** was then used in the Mitsunobu reaction employing diethyl azodicarboxylate (DEAD) and triphenylphosphine in the absence of base.[107] Elimination did not occur and the epoxides **92** and **93** were reformed with a 4:1 ratio of β:α-TBDPS epoxide (Scheme 36).

Scheme 37 Proposed mechanism to explain the observed selectivity of the Mitsunobu dehydration reaction

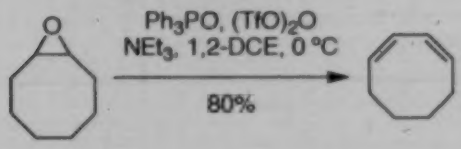


A plausible mechanism is proposed to explain the observed selectivity of the Mitsunobu dehydration reaction (Scheme 37). The Mitsunobu dehydration reaction starts with the condensation of triphenylphosphine and DEAD. The resultant anion deprotonates one of the alcohols as these are the only

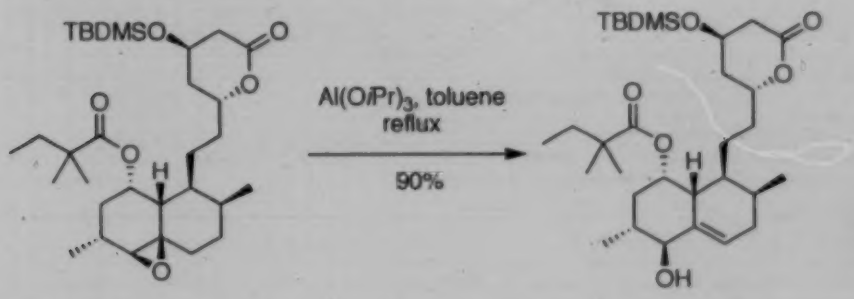
nucleophiles present.[108-111] The alcohol which is deprotonated, either C3 or C4 determines the stereochemistry of the epoxide which is formed. Depicted in Scheme 37 is deprotonation of the alcohol at C3 which would be expected to provide the β-TBDPS epoxide **92**. The alcohol at C3 is less hindered than the alcohol at C4 and thus this is a possible explanantion for the observed selectivity. As manipulation of the TBDPS diol **94** did not seem to be the proper approach, a different route was required. A literature search uncovered two promising possibilities.[112, 113]

Scheme 38 Direct elimination of epoxides to dienes (Hendrickson reaction **A**)[112] and rearrangement of an epoxide to an allylic alcohol (**B**) [113]

A. Direct elimination to diene

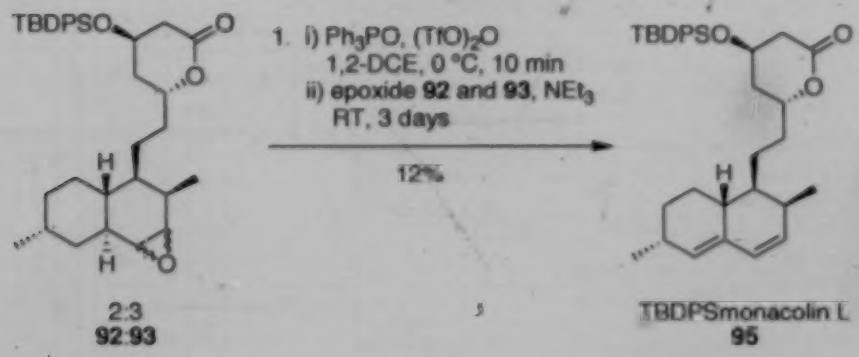


B. Rearrangement to allylic alcohol



The direct elimination of an epoxide to a diene also known as the Hendrickson reaction (**A**, Scheme 38) seemed to provide a direct route to the diene system of monacolin L (**21**) from the appropriate epoxide.[112] The second report, employing aluminum isopropoxide to form an allylic alcohol, looked like a very promising approach to 3α-3,5-hydroxy-DHL **20** (**B**, Scheme 38).[113] It seems reasonable to expect that β-TBDPS epoxide **92** and α-TBDPS epoxide **93** would provide different outcomes for these two reactions. Unfortunately all attempts to separate the β-TBDPS epoxide **92** from α-TBDPS epoxide **93** failed so the mixture was tested directly in both conditions.

Scheme 39 Results of the Hendrickson reaction with the TBDPS epoxides **92** and **93**



2:3
92:**93**

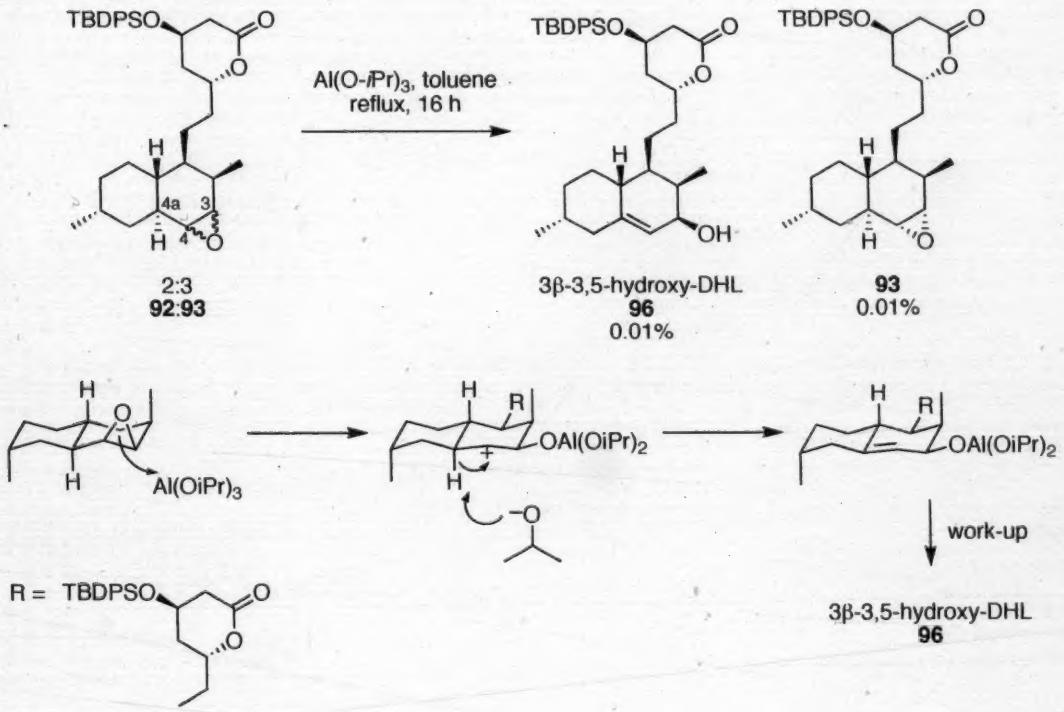
TBDPSmonacolin L
95

The Hendrickson reaction provided the TBDPSmonacolin L **95** in a yield of 12%. There are many different elimination products present and purification of TBDPSmonacolin L **95** is very difficult. There is an impurity present that could not be separated as indicated by analysis of the integration of the peaks in the ^1H

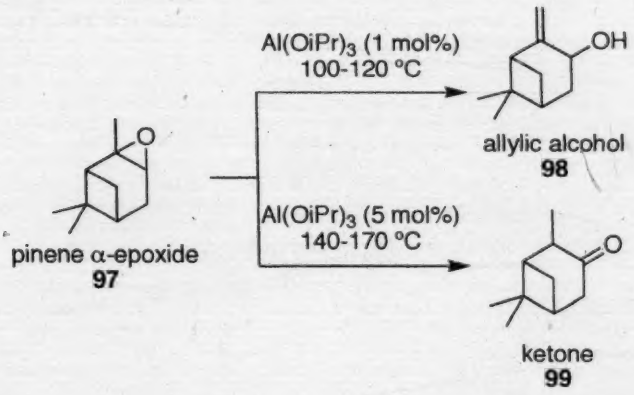
NMR spectrum of TBDPSmonacolin L **95**. It could be a product resulting from epimerization of either the C2 methyl or C6 methyl group. The desired product has three olefin proton peaks which are coupled (^{1}H COSY analysis) and exhibits chemical shifts and coupling constants which correlate closely with published data for monacolin L (**21**).[114]

The reaction of the TBDPS epoxides **92** and **93** with aluminum isopropoxide works, but it provides the product 3β-3,5-hydroxy-DHL **96** in a very low yield of 0.01 percent. Out of the many products of this reaction the only epoxide recovered from this reaction was the α-TBDPS epoxide **93**. Based on what is known about the mechanism of this reaction one is proposed in Scheme 40 to explain the formation of 3β-3,5-hydroxy-DHL **96**.[115-117] Given the stereochemistry of the alcohol at C3 of 3β-3,5-hydroxy-DHL **96**, it is reasonable to suggest that it is derived from β-TBDPS epoxide **92**. The oxophilic aluminum atom could coordinate to the oxygen of the oxirane and open the ring with subsequent deprotonation of the 4a hydrogen (Scheme 40). The stereochemistry of the 4a hydrogen is correct for elimination. In the study of the reaction of aluminum isopropoxide by Scheidl with pinene α-epoxide **97** , 1 mol% of the reagent provided the allylic alcohol **98** and 5 mol% provided the ketone **99** (Scheme 41).[115] This suggests that optimizing the temperature and amount of catalyst could provided a higher yield of the 3β-3,5-hydroxy-DHL **96**. This also suggests that some of the products of this reaction could be ketones at either C3 or C4.

Scheme 40 Results of the reaction with aluminum isopropoxide and the proposed mechanism for the reaction

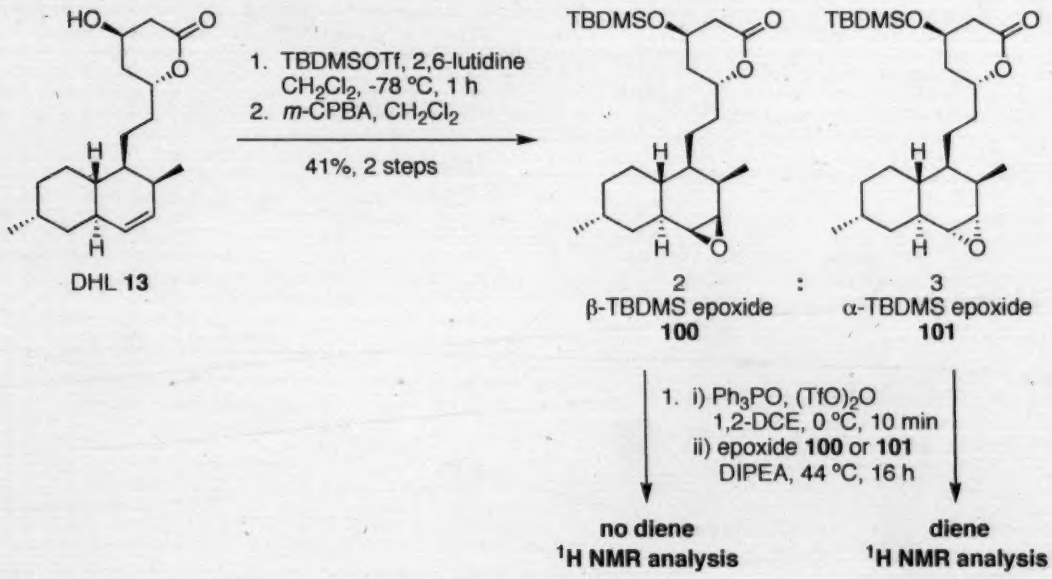


Scheme 41 Literature study on the reaction of aluminum isopropoxide with pinene α-epoxide **97**



It seemed that one of the possible reasons for having complicated mixtures of products was that epoxides were used as a mixture of stereoisomers. In an attempt to access pure stereoisomers, the protecting group was changed to the *tert*-butyldimethylsilyl group.[92, 118] The *tert*-butyldimethylsilyl group is less sterically demanding than the *tert*-butyldiphenylsilyl group. The smaller protecting group should allow for a better interaction of the epoxide compounds with silica gel and provide better separation of the epoxides.

Scheme 42 Preparation of pure stereoisomers of TBDMS epoxides **100** and **101** and preliminary test of the Hendrickson reaction with each isomer

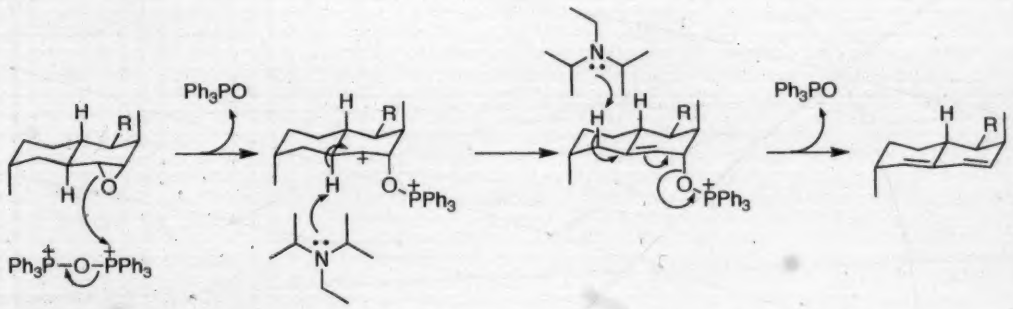


As done previously with the *tert*-butyldiphenylsilyl group, epoxidation of the *tert*-butyldimethylsilyl-protected 3-hydroxyl group provided a 2:3 mixture of β:α epoxides **100** and **101**. The subtle change of protecting group allowed Mr. Jesse Li of our group to separate **100** from **101** using flash chromatography with a 2% acetonitrile to dichloromethane gradient as the eluent. The conditions of the Hendrickson reaction were changed to provide a more rapid reaction and to hopefully increase the yield. The use of a stronger base (diisopropylethylamine, DIPEA) and elevated temperature allowed the reaction to proceed in sixteen hours rather than 3 days (Scheme 36). However, under the more forcing conditions a far more complicated mixture of products resulted and a decrease in

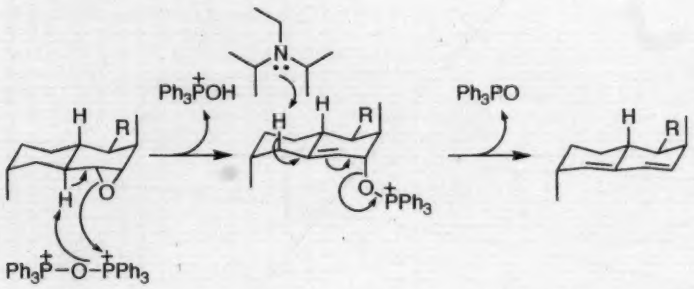
the yield of desired diene was observed. Only the α-stereoisomer led to the diene system of monacolin L (**21**) (Scheme 42). This result is consistent with what is known about the mechanism of this reaction in the literature and allows for the proposal of two plausible mechanisms as shown in Scheme 43.[119] The first mechanism, involves opening of the epoxide to a carbocation, which could potentially occur for both epoxides (**A**, Scheme 43). However, the α-TBDMS epoxide **101**, after deprotonation of H-4a has the proper stereochemistry for the second elimination to the diene across the two rings of the decalin system. The *syn*-elimination mechanism (**B**, Scheme 43) provides an explanation for the selectivity of the reaction and is in accord with the literature.[119] In both cases, the final elimination involves removal of the axial proton at C5 by the base, DIPEA, to provide the diene. This favorable stereochemical arrangement is only available for the α-epoxide.

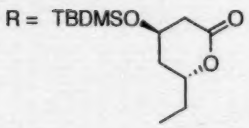
Scheme 43 Proposed mechanisms for the Hendrickson reaction of the α-TBDMS epoxide **101**

A. Cationic Mechanism



B. Syn-elimination Mechanism





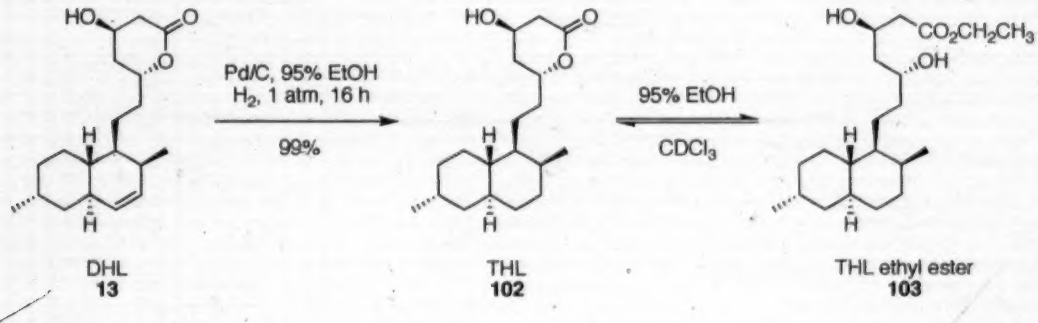
The Hendrickson reaction of the epoxides of DHL **13** provides access, for the first time, to the diene system of monacolin L (**21**). The yield of the Hendrickson reaction could be optimized by exploring different bases and temperatures.

2.7 Synthesis and Biotransformation of Tetrahydromonacolin L (102)

In considering the substrate requirements of the post-PKS enzymes, the question arose, as to whether or not the double bond of DHL **13** is a required structural element. This could be assessed rather easily, using isotopic dilution, by reducing the double bond of labeled DHL **13** followed by feeding to *A. terreus lovc*. This organism has a mutation in the *lovc* gene and is unable to produce lovastatin (**1**), but all of the post-PKS enzymes remain active and are able to produce lovastatin (**1**) when DHL **13** is added.[39, 100, 120] The desired compound, tetrahydromonacolin L (**102**, THL) was prepared as shown in Scheme 44. After reduction of the double bond if there is some ethanol still in the sample when stored the open THL ethyl ester **103** will form. However, placing the THL ethyl ester **103** in chloroform (or deuteriochloroform to monitor reaction by ^{1}H NMR) causes the lactone to reform due to traces of acid (Scheme 44). Labeled DHL (both carbon-13 and carbon-14, **106** and **104**) were prepared by feeding appropriately labeled sodium acetate to cultures of *A. nidulans lovb + lovc*.[100] By feeding carbon-14 labeled sodium acetate continuously via syringe pump the yield of ^{14}C-DHL **104** increased four times as did the total activity obtained over that achieved previously.[100] The ^{14}C-DHL **104** was diluted with ^{13}C-DHL **106** and reduced by palladium on carbon in quantitative yield to provide $^{13/14}$C-THL **105** with a total activity of 0.48 microCuries (Scheme 45). The $^{13/14}$C-THL **105** was fed to cultures of *A. terreus lovc* as was ^{13}C-DHL **106** as a control. No lovastatin (**1**) was isolated from the culture with $^{13/14}$C-THL **105**. The culture which was fed ^{13}C-

DHL **106** produced lovastatin (**1**) which means the enzymes were active. The $^{13/14}$C-THL **105** was recovered with a total activity of 0.31 microCuries.

Scheme 44 Preparation of THL **102** and equilibrium with THL ethyl ester **103**

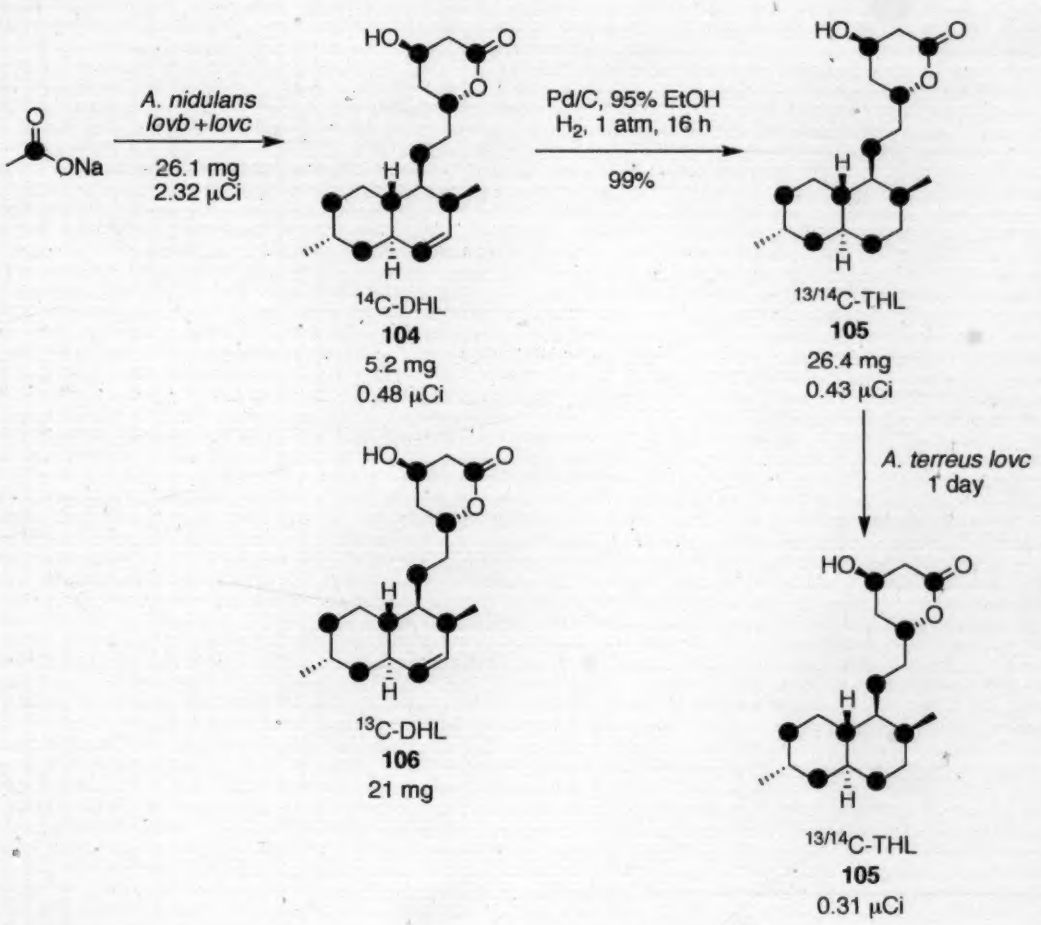


DHL
13

THL
102

THL ethyl ester
103

Scheme 45 Preparation and biotransformation results of $^{13/14}$C-THL **105**



Based on the biotransformation of $^{13/14}$C-THL **105**, we can conclude that the double bond of DHL **13** is required for recognition by the post-PKS enzymes.

2.8 Conclusions and Future Work

The research reported in this thesis has centered around the synthesis of compounds related to the biosynthesis of lovastatin (**1**) and compactin (**3**). The ability of three different ADH enzymes (YADH, HLADH and SDH) to activate a dienophile by oxidation and catalyze an intramolecular Diels-Alder reaction was explored. To this end four different substrates (decatrienol **28**, undecatrienol **29**, dodecatrienol **30** and tridecatrienol **31**) were synthesized which featured a dienophile (allylic alcohol) tethered to a diene. All three enzymes were able to activate the dienophile by oxidation to the corresponding aldehyde, although the oxidation was very slow (3 to 5 days at room temperature) and low yielding (< 5%). However, the Diels-Alder reaction occurred in solution rather than in the active site of the enzyme. A plausible explanation for this result is that the active sites were too small.

As a result of the previous study, it was discovered that IBX is able to act as both an oxidant and as a Lewis acid catalyst for the Diels-Alder reaction. The IBX oxidized undecatrienol **29** and dodecatrienol **30** to the corresponding aldehyde. The Diels-Alder reaction which ensued produced the *endo* stereoisomers (racemic) with high diastereoselectivity (indene alcohol **56** and decalin alcohol **55**).

A new lactone precursor, amide phosphonate **70**, was synthesized by using a modification of a literature route.[90] This reagent was utilized in a HEW reaction with decalin aldehyde **79** and 6-Me-decalin aldehyde **78**. Unfortunately, the yield of the reaction was low and the results were inconsistent. In addition, a labeled version, $^{13/14}$C-amide phosphonate **76**, was constructed and was unstable. It had a half life of two weeks at -20 °C. The synthesis of unlabeled desmethyl DHL **77** analog was also pursued. However, in the last step of the synthesis to form the lactone, elimination of the C3 hydroxyl group occurred and provided the α,β-unsaturated compound **82**. Due to these significant problems the synthesis of monacolin N (**76**) was not completed.

Finally, two short synthetic routes were discovered to provide access to monacolin L (**21**) and 3α-3,5-hydroxy-DHL **20**. The Hendrickson reagent was used to dehydrate TBDPS epoxides **92**(β) and **93**(α) to provide TBDPSmonacolin L **95**. The Lewis acid aluminum isopropoxide was employed to rearrange TBDPS epoxides **92**(β) and **93**(α) and yielded 3β-3,5-hydroxy-DHL **96**. While the aluminum isopropoxide reaction was very low yielding and provided the allylic alcohol with the opposite stereochemistry desired, it is possible that the alcohol of 3β-3,5-hydroxy-DHL **96** could be inverted to form 3α-3,5-hydroxy-DHL **20** after removal of the protecting group.

Optimization of both the Hendrickson reaction and the reaction employing aluminum isopropoxide is required to provide access to the putative intermediates of the post-PKS transformations of DHL **13**. As the synthetic routes are short, it should allow the synthesis of monacolin L (**21**) and 3α-3,5-hydroxy-DHL **20** in labeled form for biosynthetic studies.

CHAPTER 3. Experimental

3.1 GENERAL METHODS FOR CHEMICAL SYNTHESES

3.1.1 Reagents, Solvents and Solutions

All reactions involving air or moisture sensitive reactants were performed under a positive pressure of dry argon. Reagents and solvents were reagent grade and used as supplied unless otherwise stated. For anhydrous reactions, solvents were dried according to Amarego and Chai.[121] Tetrahydrofuran and diethyl ether were distilled over sodium and benzophenone under an atmosphere of dry argon. Acetonitrile, dichloromethane, methanol, pyridine and triethylamine were distilled over calcium hydride. Removal of solvent was performed under reduced pressure below 40 °C using a Büchi rotary evaporator, followed by evacuation (< 0.1 mm Hg) to a constant sample weight. Deionized water was obtained from a milliQ reagent water system (Millipore Co., Milford, MA). Unless otherwise specified, solutions of NH_4Cl, $NaHCO_3$, HCl, NaOH, and LiOH refer to aqueous solutions. Brine refers to a saturated aqueous solution of NaCl. Ether refers to diethyl ether.

All reagents employed were of American Chemical Society (ACS) grade or finer and were used without further purification unless otherwise stated.

3.1.2 Purification Techniques

All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC) using glass plates with a UV fluorescent indicator (normal SiO_2, Merck 60 F_{254}). One or more of the following methods were used for visualization: UV absorption by fluorescence quenching; iodine staining; (phosphomolybdic acid:ceric sulfate:sulfuric acid:H_2O/10 g:1.25 g:12 mL:238 mL) spray. Flash chromatography was performed according to the method of Still *et al.*[122] using Merck type 60, 230-400 mesh silica gel. Preparation of silver nitrate impregnated silica gel, for column chromatography and silver nitrate impregnated TLC plates was achieved using a modified procedure.[123]

High performance liquid chromatography (HPLC) was performed on either a Beckman System Gold instrument equipped with a model 166 variable wavelength UV detector and a Rheodyne 7725i injector with a 20 to 2000 μL sample loop, on a Rainin instrument equipped with a Rainin model UV-1 variable wavelength UV detector and a Rheodyne 7725i injector fitted with a 1 mL sample loop, on a Gilson instrument equipped with a model 152 variable wavelength UV detector and a Rheodyne 7010 injector fitted with a 1 mL sample loop, or on a Varian prostar equipped with a model 325 variable wavelength UV detector and a Rheodyne 7725i injector fitted with a 20 to 2000 μL sample loop. The columns used were Waters Nova-Pak cartridges (reverse phase, 8NVC18, 4 μm C_{18}

column, 60 Å, 4 μm, 8 x 100 mm), Waters μBondapak cartridges (reverse phase μBondapak, WAT037684, C_{18} column, 125 Å, 10 μm, 25 x 100 mm), Waters Nova-Pak cartridges (reverse phase, 8NVPH, 4 μm phenyl column, 60 Å, 4 μm, 8 x 100 mm) or Varian C_{18} steel walled column (reverse phase, R0086200C5, microsorb-MV100, 5μm C_{18} column, 5 μm, 4.6 x 250 mm). All HPLC solvents were filtered with a Millipore filtration system under vacuum before use. All GC-MS were performed using a Zebron DB5 30 m column of with a stationary phase thickness of (0.24 μm) using the method (50 °C → 250-290 °C @ 10 °C/min.).

3.1.3 Instrumentation for Compound Characterization

Melting points are uncorrected and were determined on a Thomas-Hoover oil immersion apparatus using open capillary tubes. Optical rotations were measured on a Perkin Elmer 241 polarimeter with a microcell (10 cm, 1 mL) at ambient temperature and are reported in units of 10^{-1} deg cm^2 g^{-1}. All specific rotations reported were referenced against air and were measured at the sodium D line and values quoted are valid within ±1 °. Infrared spectra (IR) were recorded on a Nicolet Magna 750 or a 20SX FT-IR spectrometer. Cast refers to the evaporation of a solution on a NaCl plate. Mass spectra (MS) were recorded on a Kratos AEIMS-50 (high resolution (HRMS), electron impact ionization (EI), and Micromass ZabSpec Hybrid Sector-TOF positive mode electrospray

ionization ((ES), 0.5% solution of formic acid in MeCN:H_2O/1:1) instruments. Microanalyses were obtained using a Perkin Elmer 240 or Carlo Erba 1108 elemental analyzers.

Nuclear magnetic resonance (NMR) spectra were obtained on Inova Varian 300, 400, 500 and 600 MHz spectrometers. ^{1}H NMR chemical shifts are reported in parts per million (ppm) downfield relative to tetramethylsilane (TMS) using the residual proton resonance of solvents as reference: $CDCl_3$ δ 7.24, CD_2Cl_2 δ 5.32, D_2O δ 4.72, and CD_3OD δ 3.30. ^{13}C NMR chemical shifts are reported relative to $CDCl_3$ δ 77.0, CD_2Cl_2 δ 53.8, and CD_3OD δ 49.0. Selective homonuclear decoupling, shift correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC) and attached proton test (APT) were used for signal assignments. ^{1}H NMR data are reported in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; qn, quintet; p, pentet; h, heptet and m, multiplet), number of protons, coupling constant (*J*) in Hertz (Hz) and assignment. When appropriate, the multiplicity is preceded by br, indicating that the signal was broad or app, to indicate the observed splitting pattern. All literature compounds had IR, ^{1}H NMR, and mass spectra consistent with the assigned structures. More detailed analysis of ^{1}H NMR and ^{13}C NMR spectra was performed for all unknown compounds.

Radioactivity was determined using standard liquid scintillation procedures in plastic 10 mL scintillation vials with Beckman Ready-gel scintillation cocktail. The scintillation counter used a Beckman LS 5000TD with automatic quench control to directly determine decompositions per minute (dpm) in the labeled samples against a quench curve prepared from Beckman carbon-14 quenched standards.

Chiral GC-MS analysis employed a β-DEX 120 column from Supelco Inc. The method used had a flow rate of 1.0 mL min^{-1} with an injection split of 10:1. The temperature started at 80 °C with a ramp of 1 °C min^{-1} up to 160 °C, 10 °C min^{-1} up to 200 °C and then held at 200 °C for 36 minutes to complete the run.[67]

3.1.4 Biological Media Preparations

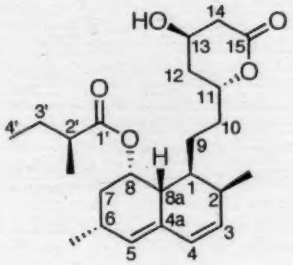
The water used for all media preparations was purified on an ELGA Maxima model water purifier and had a resistivity of 18 MΩ or greater.

Spores of *A. nidulans lovb + lovc* were propagated on potato dextrose agar, containing 0.1 g L^{-1} streptomycin sulfate, by spotting an aqueous suspension of spores with a sterile Pasteur pipette. After three weeks at 30 °C the spores were harvested by suspension in deionized water (1 mL) and rubbing the surface of the plate with a sterile glass rod. The newly suspended spores were divided into Eppendorf tubes (10 with 100 μL of solution) and stored at -20 °C until needed.

A. nidulans growth medium was prepared by dissolving glucose (20 g), yeast extract (20 g), peptone (1 g) and a solution of *para*-aminobenzoic acid (1 mL of a 1 mg mL^{-1} aqueous solution) in deionized water (1 L) in a 2L Erlenmeyer flask. *A. nidulans* production medium was prepared by the addition of trace elements solution (1 mL of a solution containing 1.0 g $FeSO_4 \cdot 7H_2O$, 8.8 g $ZnSO_4 \cdot 7H_2O$, 0.4 g $CuSO_4 \cdot 5H_2O$, 0.15 g $MnSO_4 \cdot 4H_2O$, 0.1 g $Na_2B_7O_7 \cdot 10H_2O$, 0.05 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ and 0.5 mL conc. HCl in 1 L of deionized water), 10xAMM salts solution (100 mL of a solution containing 60 g $NaNO_3$, 5.2 g KCl, 15.2 g KH_2PO_4 in 1 L of deionized water adjusted to pH 6.5), a solution of *para*-aminobenzoic acid (1 mL of a 1 mg mL^{-1} aqueous solution) and cyclopentanone (0.9 mL) to deionized water (1 L) in a 2 L Erlenmeyer flask. After this solution was sterilized, a solution of $MgSO_4 \cdot 7H_2O$ (2.5 mL of 20% solution in deionized water) and a concentrated solution of lactose (25 mL of 40% solution in deionized water) was added to the production media in the 2 L Erlenmeyer flask.

All biological media solutions and equipment were sterilized in an autoclave at 121 °C and 15 PSI of steam pressure for 15 minutes.

3.2 Experimental Data



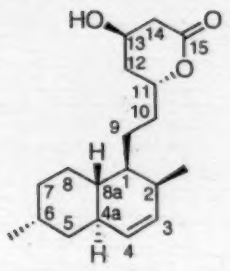
Lovastatin

(*S*)-((1*S*,3*R*,7*S*,8*S*,8a*R*)-8-(2-((2*R*,4*R*)-4-Hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl)ethyl)-3,7-dimethyl-1,2,3,7,8,8a-hexahydronapthalen-1-yl)2-methylbutanoate (1).[120]

Spores of *A. terreus lovC* were used to inoculate *A. nidulans* growth media (1 L) in a sterile, 2 L Erlenmeyer flask. The flask was sealed with a foam plug, covered with aluminum foil and incubated in a shaker at 30 °C and 200 rpm for 2 days. The mycelium was harvested by filtration through miracloth (Calbiochem), divided into 4 equal portions and transferred to *A. nidulans* production media (1 L) in a 2 L Erlenmeyer flask. Then, a solution of ^{13}C-DHL **106** (30 mg in 5 mL of EtOH) was delivered to the flask using a syringe pump over one day. This was set up so that the solution was added to the flask while it was in the incubator at 30 °C and 200 rpm. After the addition of ^{13}C-DHL **106** the mycelium was incubated for 2 days. The mycelium was removed by vacuum filtration and the filtrate was acidified with HCl (10 mL of conc. HCl) to pH 2. The aqueous media

was extracted with CH_2Cl_2 (750 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the product was purified by preparative TLC (20 cmx20 cm, 1000 micron SiO_2, 50% EtOAc:hexanes, eluted 3 times). This provided 3 mg of lovastatin (**1**) as a slightly yellow solid.

$[\alpha]_D^{25}$ 314° (*c* 1.8, CH_3CN); IR ($CHCl_3$, cast) 3435, 2963, 1725, 1258, 1187 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 5.94 (d, 1H, *J* = 9.7 Hz, H4), 5.74 (dd, 1H, *J* = 6.0, 9.7 Hz, H3), 5.34 (ddd, 1H, *J* = 3.0, 3.1, 3.1 Hz, H8), 4.59 (m, 1H, H11), 4.31 (m, 1H, H13), 3.29 (br s, 1H, OH), 2.67 (dd, 1H, *J* = 5.0, 17.7 Hz, H14), 2.59 (dd, 1H, *J* = 2.4, 17.7 Hz, H14), 2.40 (m, 1H, H6), 2.36-2.27 (m, 2H, H2,H2'), 2.23 (dd, 1H, *J* = 3.1, 12.0 Hz, H8a), 1.96-1.77 (m, 4H, H7,H10,H12), 1.68-1.57 (m, 3H, H1,H3',H12), 1.49-1.30 (m, 3H, H9,H3'), 1.25 (m, 1H, H10), 1.06 (d, 3H, *J* = 7.0 Hz, C$\underline{H}_3$2'), 1.03 (d, 3H, *J* = 7.3 Hz, C$\underline{H}_3$6), 0.85 (d, 3H, *J* = 7.0 Hz, C$\underline{H}_3$2), 0.84 (t, 3H, *J* = 7.5 Hz, H4'); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.8 (C1'), 170.8 (C15), 133.0 (C3), 131.5 (C4a), 129.5 (C5), 128.2 (C4), 76.5 (C11), 67.9 (C8), 62.4 (C13), 41.4 (C2'), 38.5 (C14), 37.2 (C8a), 36.5 (C1), 36.0 (C12), 32.9 (C10), 32.6 (C7), 30.6 (C2), 27.4 (C6), 26.7 (C3'), 24.2 (C9), 22.8 (C6$\underline{C}H_3$), 16.2 (C2'$\underline{C}H_3$), 13.8 (C2$\underline{C}H_3$), 11.6 (C4'); HRMS (ES) calcd for $C_{24}H_{37}O_5$ 405.2641, found 405.2683 (M^++H)



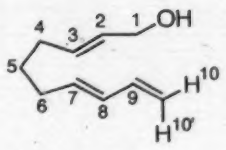
Dihydromonacolin L

(4*R*,6*R*)-6-(2-((1*S*,2*S*,4a*R*,6*R*,8a*S*)-2,6-Dimethyl-1,2,4a,5,6,7,8,8a-octahydronapthalen-1-yl)ethyl)-4-hydroxytetrahydro-2*H*-pyran-2-one (13).[100, 120, 124]

A concentrated spore suspension of *Aspergillus nidulans lovb* + *lovc* (10 μL) was used to inoculate *A. nidulans* growth media (1 L) in a sterile, 2 L Erlenmeyer flask. The flask was sealed with a foam plug, covered with aluminum foil, incubated in a shaker at 30 °C and 200 rpm for four days. The mycelium was then harvested by filtering the solution through miracloth (Calbiochem) followed by rinsing with lactose solution (2 L of a 1% solution of lactose in sterile water). The wet mycelium was then divided into four equal portions and used to inoculate *A. nidulans* production media (4x1 L) in 2 L Erlenmeyer flasks. These flasks were sealed with a foam plug, covered with aluminum foil, incubated in a shaker at 30 °C and 200 rpm for two days. Then solid, sodium acetate (1 g) was added once per day for one week. The mycelia were removed by vacuum filtration and the combined filtrate (4 L) was acidified to pH 2 with HCl (20 mL of conc. HCl). The aqueous media was then extracted with CH_2Cl_2 (2x1.5 L) and dried over

Na_2SO_4. The solvent was removed *in vacuo*. The product was purified by flash chromatography (SiO_2, isocratic elution, 40% EtOAc:hexanes) to provide DHL **13** (185 mg) as a slightly yellow solid with a typical yield of 50-70 mg L^{-1} of production media.

R_f 0.27 (60% EtOAc:hexanes); $[\alpha]_D^{25}$ 123.9° (*c* 0.5, $CHCl_3$); mp 162-163 °C; IR (microscope) 3391, 3018, 2910, 1713, 1254, 1062, 1047 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 5.57 (ddd, 1H, *J* = 2.7, 4.9, 9.8 Hz, H3), 5.28 (d, 1H, *J* = 9.8 Hz, H4), 4.67 (m, 1H, H11), 4.38 (m, 1H, H13), 2.72 (dd, 1H, *J* = 5.1, 17.6 Hz, H14), 2.60 (ddd, 1H, *J* = 1.6, 3.7, 17.6 Hz, H14), 2.21 (m, 1H, H2), 2.00 (m, 1H, H6), 1.95 (m, 1H, H12), 1.89 (m, 1H, H4a), 1.81-1.73 (m, 2H, H10,H12), 1.62 (m, 2H, H9), 1.58-1.42 (m, 5H, H5,H7,H8a,H10), 1.07 (dq, 1H, *J* = 3.7, 11.9 Hz, H8), 0.97 (dq, 1H, *J* = 3.0, 11.9 Hz, H8), 0.96 (d, 3H, *J* = 7.3 Hz, C$\underline{H}_3$6), 0.82 (d, 3H, *J* = 6.9 Hz, C$\underline{H}_3$2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.4 (C15), 132.6 (C3), 131.6 (C4), 76.1 (C11), 62.8 (C13), 41.4 (C8a), 40.0 (C8), 38.9 (C5), 38.7 (C14), 37.3 (C4a), 36.1 (C12), 33.2 (C10), 32.3 (C7), 32.0 (C2), 27.5 (C6), 23.7 (C1), 23.6 (C9), 18.2 (C$\underline{H}_3$6), 14.9 (C$\underline{H}_3$2); HRMS (EI) calcd for $C_{19}H_{30}O_3$ 306.2195, found 306.2190 (M^+)

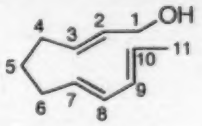


(2*E*,7*E*)-Deca-2,7,9-trien-1-ol (28).[125]

A 10 mL round bottom flask with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this flask was placed dectrienoate **39** ($1.54x10^{-3}$ mol, 300 mg) and CH_2Cl_2 (5 mL). This solution was cooled to -78 °C (dry ice/acetone) and then DIBALH ($3.4x10^{-3}$ mol, 3.4 mL of a 1 M solution in CH_2Cl_2). The solution was stirred at -78 °C for 1 hour and then quenched by the addition of NH_4Cl (3 mL) added dropwise at -78 °C. The mixture was then allowed to warm to room temperature over 1.5 hours with vigorous stirring. The solution was diluted with water (15 mL) and transferred to a 125 mL separatory funnel. The aqueous phase was extracted with CH_2Cl_2 (3x15 mL). The combined organic extracts were washed sequentially with water (15 mL), brine (15 mL) and dried over Na_2SO_4. The product was purified using flash chromatography (SiO_2, isocratic elution, 20% EtOAc:hexanes). This provided decatrienol **28** ($4.62x10^{-4}$ mol, 70 mg) as an oil in a yield of 30%.

R_f 0.22 (20% EtOAc:hexanes); IR (CH_2Cl_2 cast) 3324, 3085, 3007, 2927, 2856, 1670, 1652, 1603 cm^{-1}; GC-MS analysis (β-DEX, 80-200 °C @ 1 °C/min) 43.9 min; ^1H NMR ($CDCl_3$, 600 MHz) δ 6.31 (ddt, 1H, J = 0.33, 10.4, 17.0 Hz, H9), (app ddh, 1H, J = 0.66, 10.3, 15.2 Hz, H7), 5.72-5.63 (m, 3H, H2,H3,H8), 5.10 (ddt, 1H, J = 0.7, 1.8, 17.0 Hz, H10'), 4.97 (ddt, 1H, J = 0.55, 1.8, 10.2 Hz, H10),

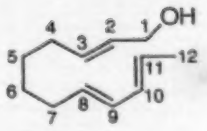
4.10 (m, 2H, H1), 2.13-2.06 (m, 4H, H4,H6), 1.50 (p, 2H, J = 7.6 Hz, H5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 137.1, 134.7, 132.8, 131.2, 129.2, 114.7, 63.7, 31.8, 31.5, 28.4; HRMS (+ES) calcd for $C_{10}H_{16}AgO$ 259.0252, found 259.0247 (M^++Ag)



(2*E*,7*E*,9*E*)-Undeca-2,7,9-trien-1-ol (29).

The procedure used was the same as that used to prepare decatrienol **28**. The amounts of reagents used were undecatrienoate **42** ($1.68x10^{-3}$ mol, 349 mg), DIBALH ($3.69x10^{-3}$ mol, 3.7 mL of 1 M solution in CH_2Cl_2) and CH_2Cl_2 (10 mL).The product was purified by flash chromatography (SiO_2, isocratic elution, 20% EtOAc:hexanes). This provided undecatrienol **29** ($1.07x10^{-3}$ mol, 179 mg) as an oil with a yield of 64%.

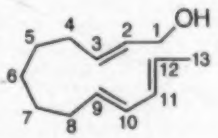
R_f 0.23 (20% EtOAc:hexanes); ^1H NMR ($CDCl_3$, 300 MHz) δ 6.04-5.91 (m, 2H, H8,H9), 5.66-5.46 (m, 4H, H2,H3,H7,H10), 3.74 (dd, 1H, J = 6.5, 6.6 Hz, H1), 3.61 (dd, 1H, J = 6.5, 6.6 Hz, H1), 2.06-1.98 (m, 4H, H4,H6), 1.69 (d, 3H, J = 6.3 Hz, H11), 1.59-1.46 (m, 2H, H5); GC-MS analysis (β-DEX, 80-200 °C @ 1 °C/min) 55.8 min



(2*E*,8*E*,10*E*)-Dodeca-2,8,10-trien-1-ol (30).

The procedure used was the same as that used to prepare decatrienol **28**. The amounts of reagents used were dodecatrienoate **50a** (1.51×10^{-3} mol, 337 mg), DIBALH (3.3×10^{-3} mol, 3.3 mL of a 1 M solution in CH_2Cl_2) and CH_2Cl_2 (10 mL). The product was purified using flash chromatography (SiO_2, isocratic elution, 20% EtOAc:hexanes). This provided dodecatrienol **30** (9.06×10^{-4} mol, 163 mg) as an oil in a yield of 60%.

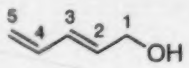
R_f 0.24 (20% EtOAc:hexanes); GC-MS analysis (β-DEX, 80-200 °C @ 1 °C/min) 67.5 min



(2*E*,9*E*,11*E*)-Trideca-2,9,11-trien-1-ol (31).

The procedure used was the same as that used to prepare decatrienol **28**. The amounts of reagents used were tridecatrienoate **51a** (1.21×10^{-3} mol, 285 mg), DIBALH (2.0×10^{-3} mol, 2.0 mL of a 1 M solution in CH_2Cl_2) and CH_2Cl_2 (5 mL). The product was purified using flash chromatography (SiO_2, isocratic elution, 20% EtOAc:hexanes). This provided tridecatrienol **31** (7.87×10^{-4} mol, 153 mg) as an oil in a yield of 65%.

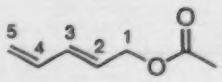
R_f 0.25 (20% EtOAc:hexanes); GC-MS analysis (β-DEX, 80-200 °C @ 1 °C/min) 60.6 min



(*E*)-Penta-2,4-dien-1-ol (33).[68, 70]

Into a 25 mL round bottom flask with a magnetic stirrer bar was placed 1,4-pentadien-3-ol (**32**) (0.010 mol, 1 mL) and THF (1 mL). This solution was cooled to 0 °C and then a 1:1 solution of conc. H_2SO_4:H_2O was added via syringe. This mixture was stirred at 0 °C for one hour and then at room temperature for 24 hours. The reaction mixture was transferred to an Erlenmeyer flask and neutralized with solid $NaHCO_3$. The resultant brown, biphasic mixture was placed into a 250 mL separatory funnel and extracted with Et_2O (3x15 mL). The combined ethereal extracts were washed with saturated $NaHCO_3$ until the pH of the washings were 7. The organic extracts were then washed with brine (2x30 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo*. The product was purified by flash chromatography (SiO_2, isocratic elution, 30% Et_2O:pentane). This provided (*E*)-penta-2,4-dien-1-ol (**33**) ($6x10^{-3}$ mol, 532 mg) as a clear oil in a yield of 62%.

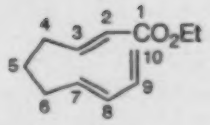
R_f 0.16 (20% EtOAc:hexanes); ^1H NMR ($CDCl_3$, 300 MHz) δ 6.40-6.18 (m, 2H, H3,H4), 5.83 (dt, 1H, J = 5.8, 14.6 Hz, H2), 5.23-5.16 (m, 1H, H5), 5.10-5.05 (m, 1H), 4.18-4.15 (m, 2H, H1)



(*E*)-Penta-2,4-dienyl acetate (34).[69]

A 250 mL round bottom flask with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this flask was placed (*E*)-penta-2,4-dienol (**33**) (0.065 mol, 5.47 g), NEt_3 (0.098 mol, 13.7 mL) and THF (100 mL). The flask was cooled to 0 °C and acetyl chloride (0.098 mol, 7 mL) was added slowly over 5 minutes. The reaction mixture was then stirred at room temperature for 2 hours. Water was then added to dissolve the white precipitate. The mixture was transferred to a 500 mL separatory funnel. The aqueous solution was extracted with CH_2Cl_2 (3x50 mL). The combined organic extracts were washed sequentially with water (75 mL), brine (75 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo*. The product was then purified using flash chromatography (SiO_2, isocratic elution, 5% EtOAc:hexanes) to provide (*E*)-penta-2,4-dienyl acetate (**34**) (0.031 mol, 3.87 g) as a clear oil in a yield of 48%. According to the ^1H NMR spectrum, there was 3% of the (*Z*)-penta-2,4-dienyl acetate present. The data presented is for (*E*)-penta-2,4-dienyl acetate (**34**).

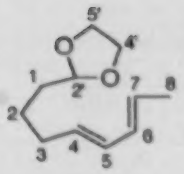
R_f 0.60 (20% EtOAc:hexanes); IR (CH_2Cl_2 cast) 3089, 3009, 2944, 1743, 1606 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.36-6.24 (m, 2H, H3,4), 5.78-5.72 (m, 1H, H2), 5.26-5.21 (m, 1H, H5), 5.14-5.11 (m, 1H, H5), 4.58 (d, 2H, J = 6.6 Hz, H1), 2.05 (s, 3H, $C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.7, 135.9, 134.7, 127.0, 118.6, 64.5, 20.9; HRMS (EI) calcd for $C_7H_{10}O_2$ 126.0681, found 126.0679 (M^+)



(2*E*,7*E*)-Ethyl deca-2,7,9-trienoate (39).[125]

The procedure followed was the same as that used prepare undecatriene **42**. The amounts of reagents used were octadienal **37** (0.012 mol, 1.49 g), Wittig reagent **38** (0.014 mol, 5.0 g) and CH_2Cl_2 (80 mL). The product was purified using flash chromatography (SiO_2, isocratic elution, 2% Et_2O:pentane). This provided decatrienoate **39** ($3.6x10^{-3}$ mol, 700 mg) as a volatile oil in a yield of 30%.

R_f 0.26 (5% EtOAc:hexanes); IR (CH_2Cl_2 cast) 2981, 2933, 1721, 1654, 1602 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.94 (dt, 1H, J = 15.4, 6.9 Hz, H3), 6.30 (dt, 1H, J = 16.9, 10.2 Hz, H8), 6.10-6.00 (m, 1H, H9), 5.80 (dt, 1H, J = 15.7, 1.6 Hz, H7), 5.66 (dt, 1H, J = 15.4, 7.0 Hz, H2), 5.14-5.04 (m, 1H, H10), 5.00-4.94 (m, 1H, H10), 4.17 (q, 2H, J = 7.1 Hz, $OC\underline{H}_2CH_3$), 2.21 (dq, 2H, J = 1.6, 7.2 Hz, H6), 2.11 (q, 2H, J = 7.0 Hz, H4), 1.56 (app p, 2H, J = 7.5 Hz, H5), 1.27 (t, 3H, J = 7.1 Hz, $OCH_2C\underline{H}_3$); HRMS (EI) calcd for $C_{12}H_{18}O_2$ 194.1307, found 194.1312 (M^+)



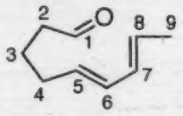
2-((4*E*,6*E*)-Octa-4,6-dienyl)-1,3-dioxolane (40).[78, 126]

A 100 mL round bottom flask with a magnetic stirrer bar was fitted with a condenser and flame dried under Ar. Into this flask was placed magnesium turnings (0.05 mol, 1.2 g) which were ground briefly with a mortar and pestle. A small amount of THF was added to just cover the magnesium followed by 2-(2-bromoethyl)-1,3-dioxolane (**35**) (0.04 mol, 5.1 mL) added in one portion. This mixture was stirred vigorously under Ar and heated if necessary to initiate the reaction. Once the reaction was self-sustaining (boiling without external heating) the remaining THF was added to achieve a final volume of 45 mL. A gentle reflux was maintained by controlling the rate of reaction with an ice bath. After 2 hours the reaction was complete and the contents of the flask were transferred rapidly via cannula (positive pressure of Ar) to a 60 mL addition funnel. The addition funnel was attached to a 250 mL round bottom flask and both were previously, flame dried under Ar. The 250 mL round bottom flask contained a magnetic stir bar, (2*E*,4*E*)-hexa-2,4-dienyl acetate (0.036 mol, 5.3 mL), Li_2CuCl_4 ($1.78x10^{-3}$ mol, 17.9 mL of a freshly prepared, 0.1 M solution in THF) and THF (17.1 mL). The 250 mL round bottom flask was cooled to -15 °C (dry ice:ethylene glycol) and the Grignard reagent was added dropwise over 1 hour with vigorous stirring under Ar. A successful reaction was indicated by a final color of purple in

the reaction mixture. Once addition of the Grignard reagent was complete, the reaction mixture was stirred for another 3 hours at -15 °C. To quench the reaction, NH_4Cl (15 mL) was added, the mixture was stirred vigorously and allowed to warm to room temperature (1 hour). The reaction mixture was diluted with water (100 mL) and transferred to a 500 mL separatory funnel. The mixture was extracted with Et_2O (2x100 mL). The combined organic extracts were washed sequentially with water (100 mL), brine (100 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo*. The resultant light, yellow oil was taken up in a 3:1 solution of CH_3OH:H_2O (100 mL) and NaOH (12 mL of a 2 M solution) was added to hydrolyze unreacted (2*E*,4*E*)-hexa-2,4-dienyl acetate. This mixture was stirred vigorously at room temperature and monitored by TLC. After 1.5 hours the reaction was stopped and the contents of the flask were transferred to a 500 mL separatory funnel. The aqueous solution was extracted with Et_2O (3x100 mL). The combined organic extracts were washed with brine (150 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo*. The α-substituted product **40** was separated from the γ-substituted by flash chromatography ($AgNO_3$ impregnated SiO_2, isocratic elution, 5% Et_2O:pentane) to provide both products as clear oils. The α-substituted product **40** (0.011 mol, 2.03 g) was obtained in a yield of 31%. The γ-substituted product ($9.1x10^{-4}$ mol, 165 mg) was obtained in a yield of 3%. The data presented is for the α-substituted product **40**.

R_f 0.28 (10% EtOAc:hexanes); IR (microscope) 3016, 2950, 2881, 1634 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.06-5.95 (m, 2H, H5,6), 5.65-5.46 (m, 2H, H4,7), 4.85

(t, 1H, J = 4.6 Hz, H2'), 3.98-3.81 (m, 4H, H4',5'), 2.10 (q, 2H, J = 7.1 Hz, H3), 1.73 (d, 3H, J = 6.0 Hz, H8), 1.71-1.62 (m, 2H, H2), 1.57-1.46 (m, 2H, H2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 131.6 (C6), 131.2 (C4), 130.7 (C5), 126.9 (C7), 104.5 (C2'), 64.8 (C4',5'), 33.3 (C1), 32.3 (C3), 23.7 (C2), 17.9 (C8); HRMS (EI) calcd for $C_{11}H_{18}O_2$ 182.1307, found 182.3036 (M^+)

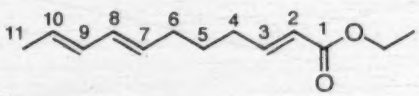


(5*E*,7*E*)-Nona-5,7-dienal (41).[69, 78]

Into a 250 mL round bottom flask was placed octadiene dioxolane **40** (0.11 mol, 2.03 g), THF (65 mL), water (60 mL) and glacial AcOH (30 mL). The flask was fitted with a condenser and heated at reflux for 8 hours. The reaction mixture was allowed to cool to room temperature (1 hour) and then the crude mixture was transferred to a 1 L Erlenmeyer flask. The solution was diluted with water (200 mL) and neutralized with solid $NaHCO_3$ (58 g). The neutralized, aqueous solution was then transferred to 1 L separatory funnel and extracted with pentane (3x100 mL). The combined organic extracts were washed sequentially with water (100 mL), brine (100 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo*. Then, the aldehyde was purified using column chromatography (SiO_2, isocratic elution no pressure, 100% CH_2Cl_2) which provided (5*E*,7*E*)-nona-5,7-dienal (**41**) (0.11 mol, 1.5 g) as a volatile clear oil in quantitative yield.

R_f 0.50 (100% CH_2Cl_2); IR (microscope) 3016, 2933, 2883, 2720, 1726, 1626

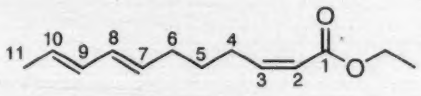
cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 9.76 (t, 1H, J = 1.8 Hz, H1), 6.06-5.96 (m, 2H, H6,7), 5.69-5.55 (m, 1H, H8), 5.48-5.14 (m, 1H, H5), 2.43 (dt, 2H, J = 7.2, 1.8 Hz, H2), 2.10 (q, 2H, J = 6.9 Hz, H4), 1.78-1.68 (m, 5H, H3,9); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 202.4 (C1), 131.5 (C6,7), 131.3 (C6,7), 130.1 (C5), 127.6 (C8), 43.2 (C2), 31.8 (C4), 21.8 (C3), 18.0 (C9); HRMS (EI) calcd for $C_9H_{14}O$ 138.1045, found 138.1045 (M^+)



(2*E*,7*E*,9*E*)-Ethyl undeca-2,7,9-trienoate (42).

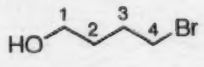
A 250 mL round bottom flask with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this flask was placed Wittig reagent **38** (0.016 mol, 5.40 g) and CH_2Cl_2. This mixture was stirred at room temperature under Ar until the Wittig reagent **38** was fully dissolved. Then, diene aldehyde **41** (0.013 mol, 1.79 g) was added in one portion. The mixture was stirred overnight at room temperature under Ar. The CH_2Cl_2 was removed *in vacuo* and the resultant, viscous oil was adsorbed onto SiO_2. The mixture of *E* and *Z* isomers were separated by flash chromatography (SiO_2, gradient elution, 2% Et_2O:pentane, 4% Et_2O:pentane, 5% Et_2O:pentane) to yield (2*E*,7*E*,9*E*)-ethyl undeca-2,7,9-trienoate (**42**) (5.83×10^{-3} mol, 1.21 g) and (2*Z*,7*E*,9*E*)-ethyl undeca-2,7,9-trienoate (**43**) (2.73×10^{-4} mol, 56.8 mg) as clear, volatile oils in yields of 45 and 2% respectively.

R_f 0.50 (10% EtOAc:hexanes) ; IR (CH_2Cl_2 cast) 3016, 2981, 2931, 2855, 1721, 1654 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.95 (dt, 1H, *J* = 15.6, 6.6 Hz, H3), 6.07-5.95 (m, 2H, H8,H9), 5.81 (dt, 1H, *J* = 15.6, 1.5 Hz, H2), 5.67-5.43 (m, 2H, H7, H10), 4.18 (q, 2H, *J* = 7.1 Hz, $OC\underline{H}_2CH_3$), 2.24-2.16 (m, 2H, H4), 2.08 (q, 2H, *J* = 7.2 Hz, H6), 1.73 (d, 3H, *J* = 6.6 Hz, H11), 1.60-1.50 (m, 2H, H5), 1.29 (t, 3H, *J* = 7.1 Hz, $OCH_2C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.8 (C1), 149.0 (C3), 131.9 (C9), 131.1 (C8), 130.7 (C7), 127.4 (C10), 121.7 (C2), 60.6 ($O\underline{C}H_2CH_3$), 31.5 (C6), 31.0 (C4), 28.1 (C5), 17.9 (C11), 13.8 ($OCH_2\underline{C}H_3$); HRMS (EI) calcd for $C_{13}H_{20}O_2$ 208.1463, found 208.1454 (M^+)



(2*Z*,7*E*,9*E*)-Ethyl undeca-2,7,9-trienoate (43).

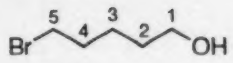
R_f 0.60 (10% EtOAc:hexanes); IR (CH_2Cl_2 cast) 3016, 2927, 2855, 1721, 1644 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.20 (dt, 1H, *J* = 11.7, 7.5 Hz, H3), 6.07-5.95 (m, 2H, H8,H9), 5.76 (dt, 1H, *J* = 11.7, 1.5 Hz, H2), 5.63-5.49 (m, 2H, H7,H10), 4.17 (q, 2H, *J* = 7.2 Hz, $OC\underline{H}_2CH_3$), 2.70-2.62 (m, 2H, H4), 2.10 (q, 2H, *J* = 7.2 Hz, H6), 1.72 (d, 3H, *J* = 6.0 Hz, H11), 1.53 (m, 2H, H5), 1.29 (t, 3H, *J* = 7.2 Hz, $OCH_2C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.3 (C1), 150.1 (C3), 131.7 (C8,9), 131.2 (C7), 130.7 (C8,9), 127.1 (C10), 119.9 (C2), 59.8 ($O\underline{C}H_2CH_3$), 32.6 (C6), 29.4 (C5), 28.4 (C4), 17.9 (C11), 13.8 ($OCH_2\underline{C}H_3$); HRMS (EI) calcd for $C_{13}H_{20}O_2$ 208.1463, found 208.1455 (M^+)



4-Bromobutan-1-ol (44a).[72]

Into a 500 mL round bottom flask was placed 1,4-butanediol (**44**) (0.17 mol, 15 mL), hydrobromic acid (0.17 mol, 19 mL of 48% aqeous solution), benzene (250 mL) and a magnetic stirrer bar. The reaction flask was attached to a Dean-Stark apparatus and heated to reflux temperature. The reaction was complete after 4 hours of reflux or 1 hour after water stopped collecting in the trap. The reaction mixture was allowed to cool to room temperature (~1 hour). Then, the contents of the reaction flask were transferred to a 500 mL separatory funnel. The organic layer was washed sequentially with 6M NaOH (100 mL), 10% HCl (100 mL), water (2x200 mL), brine (150 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* to give a dark brown oil. The product was purified by flash chromatography (SiO_2 30% EtOAc:hexanes isocratic elution) and 4-bromobutan-1-ol (**44a**) (0.08 mol, 11.6 g) was obtained as a clear oil in a yield of 45%.

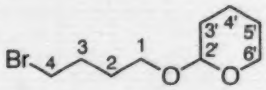
R_f 0.20 (30% EtOAc:hexanes); IR (microscope) 3332, 2941, 2876 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 3.71 (t, 2H, J = 6.0 Hz, H1), 3.47 (t, 2H, J = 7.0 Hz, H4), 2.16 (br s, 1H, OH), 2.00-1.94 (m, 2H, H3), 1.76-1.70 (m, 2H, H2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 62.0 (C1), 33.6 (C4), 31.1 (C3), 29.2 (C2)



5-Bromopentan-1-ol (45a).[72]

Into a 500 mL round bottom flask was placed 1,5-pentane diol (**45**) (0.10 mol, 10.5 mL), hydrobromic acid (0.10 mol, 12.5 mL of 48% aqueous solution), benzene (200 mL) and a magnetic stirrer bar. The procedure followed was the same as that used to prepare 4-bromobutan-1-ol (**44a**). However, the crude product was sufficiently pure to be used directly in the next reaction without purification. The product, 5-bromopentan-1-ol (**45a**), was obtained as brown oil (0.06 mol, 9.99 g) in a yield of 60%.

R_f 0.40 (50% petroleum Et_2O:EtOAc); IR (microscope) 3327, 2937, 2864 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 3.66 (t, 2H, J = 6.0 Hz, H1), 3.42 (t, 2H, J = 7.0 Hz, H5), 1.93-1.87 (m, 2H, H4), 1.63-1.57 (m, 2H, H2), 1.56-1.49 (m, 2H, H3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 62.6, 33.7, 32.5, 21.8, 24.4; HRMS (EI) calcd for $C_5H_{11}{}^{79}BrO$ 165.9993, found 164.9915 (M^+-H)

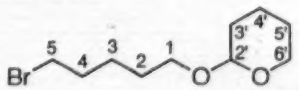


2-(4-Bromobutoxy)tetrahydro-2*H*-pyran (46).[127]

A 100 mL round bottom flask with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this flask was placed 4-bromobutan-1-ol (**44a**) (0.015 mol, 2.51 g) and CH_2Cl_2 (20 mL). The solution was cooled to 0 °C. Then 3,4-dihydropyran (0.018 mol, 1.6 mL) was added slowly followed by *p*-

TsOH ($2.62x10^{-5}$ mol, 5 mg). The mixture was stirred for 2 hours during which time it was allowed to warm to room temperature. The reaction was quenched by the addition of NEt_3 (0.1 mL). The mixture was transferred to a 125 mL separatory funnel and washed successively with water (20 mL), brine (20 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo*. The protected alcohol, 2-(4-bromobutoxy)tetrahydro-2*H*-pyran (**46**) was purified by short path distillation to give a pale yellow oil (0.012 mol, 2.94 g) in a yield of 78%.

R_f 0.64 (20% EtOAc:hexanes); IR ($CHCl_3$ cast) 2942, 2870 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.57 (1H, dd, J = 2.9, 4.2 Hz, H2'), 3.89-3.67 (m, 2H, H1,6'), 3.54-3.38 (m, 2H, H1,6'), 3.46 (t, 2H, J = 6.8 Hz, H4), 2.06-1.92 (m, 2H, H3), 1.89-1.65 (m, 5H, H2,3',4',5'), 1.64-1.46 (m, 3H, H3',4',5'); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 98.8 (C2'), 66.5 (C6'), 62.3 (C1), 33.7 (C4), 30.7, 29.8, 28.4, 25.5, 19.6; HRMS (EI) calcd for $C_9H_{16}O_2{}^{81}Br$ and $C_9H_{16}O_2{}^{79}Br$ 237.0313 and 235.0337, found 237.0310 and 235.0331 respectively (M^+-H)

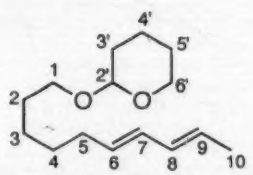


2-(5-Bromopentyloxy)tetrahydro-2*H*-pyran (47).[128]

A 250 mL round bottom flask with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this flask was placed 5-bromopentan-1-ol (**45a**) (0.06 mol, 9.99 g) and CH_2Cl_2 (150 mL). This solution was then cooled to 0 °C. Then, 3,4-dihydropyran (0.07 mol, 6.5 mL) was added slowly to the stirred solution followed by *p*-TsOH ($6.31x10^{-5}$ mol, 12 mg). The reaction mixture was

stirred overnight at room temperature. The reaction was stopped by the addition of NEt_3 (0.15 mL) and then transferred to a 500 mL separatory funnel. The CH_2Cl_2 layer was washed sequentially with water (100 mL), brine (100 mL) and dried over Na_2SO_4. The product was purified by short path distillation to provide 2-(5-bromopentyloxy)tetrahydro-2*H*-pyran (**47**) (0.03 mol, 6.92 g) in a yield of 46%.

R_f 0.60 (20% EtOAc:hexanes); IR (CH_2Cl_2 cast) 2940, 2867 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.58 (dd, 1H, *J* = 3.0, 4.5 Hz, H2'), 3.89-3.84 (m, 1H, H1), 3.75 (ddd, 1H, *J* = 6.5, 6.5, 10.0 Hz, H6'), 3.53-3.48 (m, 1H, H1), 3.42 (t, 2H, *J* = 7.0 Hz, H5), 3.41-3.38 (m, 1H, H6'), 1.93-1.88 (m, 2H, H4), 1.86-1.80 (m, 1H, H4'), 1.74-1.69 (m, 1H, H4'), 1.66-1.50 (m, 6H, H2,H3,H3',H5'); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 98.9 (C2'), 67.2 (C6'), 62.4 (C1), 33.7 (C5), 32.6 (C4), 30.7 (C4'), 28.9 (C5'), 25.5 (C3'), 25.0 (C3), 19.7 (C2); HRMS (EI) calcd for $C_{10}H_{18}O_2{}^{81}Br$ and $C_{10}H_{18}O_2{}^{79}Br$ 251.0470 and 249.0490, found 251.0472 and 249.0489 (M^+-H)

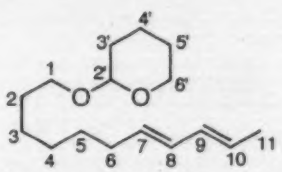


2-((6*E*,8*E*)-Deca-6,8-dienyloxy)tetrahydro-2*H*-pyran (48).[129]

The procedure followed was the same as that reported for the preparation of 2-((4*E*,6*E*)-octa-4,6-dienyl)-1,3-dioxolane (**40**). For this preparation the amounts of reagents used were 4-bromo-THP (**46**) (0.041 mol, 9.8 g), Mg (0.050 mol, 1.21 g), (2*E*,4*E*)-hexa-2,4-dienyl acetate (0.0342 mol, 5.1 mL) and Li_2CuCl_4 (1.7x10-3

mol, 17 mL of a 0.1 M solution in THF). The products of the reaction were purified by flash chromatography ($AgNO_3$•SiO_2, isocratic elution, 2% Et_2O:pentane). This provided 2-((6*E*,8*E*)-deca-6,8-dienyloxy)tetrahydro-2*H*-pyran (**48**) (0.013 mol, 3.15 g) as a clear oil in a yield of 40%. The product from the S_N2' side reaction, (*E*)-2-(5-vinyloct-6-enyloxy)tetrahydro-2*H*-pyran (**52**) (2.52×10^{-3} mol, 600 mg) was isolated as a clear oil in a yield of 7%. The data presented is for 2-((6*E*,8*E*)-deca-6,8-dienyloxy)tetrahydro-2*H*-pyran (**48**).

R_f 0.44 (10% Et_2O:pentane, $AgNO_3$•SiO_2) 0.64 (10% EtOAc:hexanes, SiO_2); IR (CH_2Cl_2 cast) 3015, 2938, 2870, 2733, 2657, 1662 cm^{-1}; ^1H NMR ($CDCl_3$ 500 MHz) δ 6.05-5.96 (m, 2H, H7,H8), 5.61-5.52 (m, 2H, H6,H9), 4.57 (dd, 1H, *J* = 2.7, 4.2 Hz, H2'), 3.89-3.85 (m, 1H, H1), 3.73 (dt, 1H, *J* = 9.6, 6.9 Hz, H6'), 3.52-3.48 (m, 1H, H1), 3.38 (dt, 1H, *J* = 9.6, 6.7 Hz, H6'), 2.06 (q, 2H), 1.87-1.80 (m, 1H), 1.74-1.68 (m, 1H), 1.73 (d, 3H, *J* = 6.5 Hz, H10), 1.63-1.50 (m, 4H), 1.44-1.22 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 131.9, 131.6, 130.3, 126.7, 98.8, 67.5, 67.3, 62.29, 62.25, 32.4, 31.8, 30.7, 29.6, 29.2, 25.7, 25.5, 19.7, 19.4, 17.9, 13.9; HRMS (EI) calcd for $C_{15}H_{26}O_2$ 238.1933, found 238.1931 (M^+)

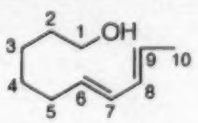


2-((7*E*,9*E*)-Undeca-7,9-dienyloxy)tetrahydro-2*H*-pyran (49).[130]

The procedure used was the same as that used to prepare 2-((4*E*,6*E*)-octa-4,6-dienyl)-1,3-dioxolane (**40**). For this preparation the amounts of reagents used

were 5-bromo-THP **47** (0.0278 mol, 5 mL), Mg (0.033 mol, 810 mg), (2*E*,4*E*)-hexa-2,4-dienyl acetate (0.023 mol, 3.5 mL) and Li_2CuCl_4 ($1.16x10^{-3}$ mol, 11.6 mL of a 0.1 M solution in THF). The products of the reaction were purified by flash chromatography ($AgNO_3$•SiO_2, isocratic elution, 5% Et_2O:pentane). This provided 2-((7*E*,9*E*)-undeca-7,9-dienyloxy)tetrahydro-2*H*-pyran (**49**) (0.012 mol, 3.04 g) as a clear oil in a yield of 43%.

R_f 0.67 (10% Et_2O:pentane, $AgNO_3$•SiO_2); IR (CH_2Cl_2 cast) 3015, 2936, 2856, 1454 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 6.04-5.97 (m, 2H, H8,H9), 5.61-5.51 (m, 2H, H7,H10), 4.58 (dd, 1H, *J* = 2.8, 4.4 Hz, H2'), 3.89-3.85 (m, 1H, H1), 3.73 (dt, 1H, *J* = 9.6, 6.9 Hz, H6'), 3.52-3.48 (m, 1H, H1), 3.38 (dt, 1H, *J* = 9.6, 6.9 Hz, H6'), 2.05 (q, 2H, *J* = 7.0 Hz, H6), 1.87-1.80 (m, 1H), 1.75-1.69 (m, 4H), 1.62-1.50 (m, 6H), 1.41-1.30 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 132.0, 131.6, 130.2, 126.6, 98.7, 67.5, 62.2, 32.4, 30.7, 29.6, 29.3, 28.9, 26.0, 25.4, 19.6, 17.9; HRMS (EI) calcd for $C_{16}H_{28}O_2$ 252.2089, found 252.2086 (M^+)

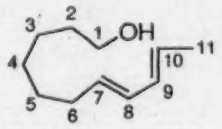


(6*E*,8*E*)-Deca-6,8-dien-1-ol (48a).[129]

Into a 50 mL round bottom flask with a magnetic stirrer bar was placed decadiene-THP **48** (0.013 mol, 3.15 g), CH_3OH (25 mL) and *p*-TsOH ($5.26x10^{-5}$ mol, 10 mg). The mixture was stirred overnight at room temperature. The solution was taken up in pentane (150 mL) and washed successively with water

(2x50 mL), brine (2x50 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo*. The product was purified by flash chromatography (SiO_2, gradient elution, 5% Et_2O:pentane, 10% Et_2O:pentane, 100% Et_2O). This provided (6*E*,8*E*)-deca-6,8-dien-1-ol (**48a**) (7.2x10-3 mol, 1.11 g) as an oil in a yield of 55%.

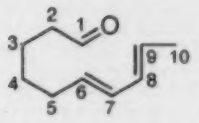
R_f 0.12 (20% EtOAc:hexanes); IR (CH_2Cl_2 cast) 3332, 3015, 2931, 2856, 1436 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.06-5.94 (m, 2H, H7,H8), 5.63-5.49 (m, 2H, H6,H9), 3.63 (t, 2H, J = 6.5 Hz, H1), 2.06 (q, 2H, J = 6.9 Hz, H5), 1.72 (d, 3H, J = 6.2 Hz, H10), 1.61-1.31 (m, 6H, H2,H3,H4); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 131.7, 131.6, 130.4, 126.8, 62.9, 32.6, 32.4, 29.1, 25.2, 17.9; LRMS (CI) calcd for $C_{10}H_{22}NO^+$ 172.2, found 172.3 (M^++NH_4)



(7*E*,9*E*)-Undeca-7,9-dien-1-ol (49a).[130]

The procedure used was the same as that for the preparation of (6*E*,8*E*)-deca-6,8-dien-1-ol (**48a**). For this preparation the amounts of reagents used were undecadiene-THP **49** (7.20x10^{-4} mol, 182 mg), CH_3OH (5 mL) and *p*-TsOH (8.99x10^{-5} mol, 17.1 mg). The reaction was quenched with NEt_3 (9.33x10^{-5} mol, 13 μL). The product was purified by flash chromatography (SiO_2, gradient elution, 5% Et_2O:pentane, 10% Et_2O:pentane, 100% Et_2O). This provided undecadiene-ol **49a** (3.6x10^{-4} mol, 60.8 mg) as a clear, volatile oil in a yield of 50%.

R_f 0.06 (10% Et_2O:pentane); IR (CH_2Cl_2 cast) 3331, 3015, 2929, 2855, 1435 cm^{-1}; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 131.9, 131.6, 130.3, 126.7, 63.0, 32.7, 32.4, 29.3, 28.9, 25.6, 17.9; LRMS (+ES) calcd for $C_{11}H_{24}NO$ 186.2, found 186.2 (M^++NH_4)

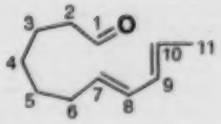


(6*E*,8*E*)-Deca-6,8-dienal (50).[69, 129]

Into a 100 mL round bottom flask with a magnetic stirrer bar was placed IBX (0.016 mol, 4.4 g), and DMSO (50 mL). The solution was stirred at room temperature for 30 minutes or until the IBX was completely dissolved. Then, (6*E*,8*E*)-deca-6,8-dien-1-ol (**48a**) (0.012 mol, 1.85 g), was added in one portion. The solution was stirred for one hour to ensure complete reaction. Then the solution was cooled to 0 °C and water (50 mL) was added to quench the reaction. The white precipitate was removed by vacuum filtration and washed with Et_2O. The filtrate was transferred to a 250 mL separatory funnel and extracted with Et_2O (3x50 mL). The combined ethereal extracts were washed sequentially with water (3x20 mL), brine (50 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the crude residue was passed through a small plug of silica gel (eluted with Et_2O). The solvent was then removed *in vacuo* and the aldehyde **50** was used directly in the following reaction as it was quite volatile.

R_f 0.36 (10% Et_2O:pentane); IR ($CHCl_3$ cast) 2955, 2926, 2857, 1727, 1458 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.75 (t, 1H, J = 1.8 Hz, H1), 6.04-5.94 (m, 2H,

H7,H8), 5.62-5.46 (m, 2H, H6,H9), 2.41 (dt, 2H, J = 7.3, 1.8 Hz, H2), 2.06 (q, 2H, J = 7.2 Hz, H5), 1.71 (d, 3H, J = 6.2 Hz, H10), 1.63 (p, 2H, J = 7.3 Hz, H3), 1.45-1.32 (m, 2H, H4)

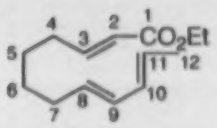


(7*E*,9*E*)-Undeca-7,9-dienal (51).[130]

A 250 mL round bottom flask with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this flask was placed Dess-Martin periodinane (7.12×10^{-2} mol, 2.41 g) and CH_2Cl_2 (55 mL). Then, a solution of undecadiene-ol **49a** (5.83×10^{-2} mol, 0.981 g) in CH_2Cl_2 (30 mL) was added slowly via syringe. The reaction mixture was stirred at room temperature overnight. The reaction was quenched by the addition of NaOH (55 mL of a 1.3 M solution) followed by stirring at room temperature for 30 minutes. The crude mixture was transferred to a 250 mL separatory funnel, the CH_2Cl_2 layer was removed and dried over Na_2SO_4. The product was purified by flash chromatography (SiO_2, isocratic elution, 10% Et_2O:pentane). This provided undecadienal **51** (3.08×10^{-2} mol, 512 mg) as a volatile oil in a yield of 53%.

R_f 0.36 (10% Et_2O:pentane); IR ($CHCl_3$ cast) 3015, 2930, 2856, 2719, 1726, 1627, 1585, 1437 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.75 (t, 1H, J = 1.8 Hz, H1), 6.04-5.94 (m, 2H, H8,H9), 5.61-5.48 (m, 2H, H7,H10), 2.41 (dt, 2H, J = 7.3, 1.8

Hz, H2), 2.07-2.02 (m, 2H), 1.72 (d, 3H, J = 6.7 Hz, H11), 1.66-1.56 (m, 2H), 1.42-1.20 (m, 4H); HRMS (EI) calcd for $C_{11}H_{18}O$ 166.1358, found 166.1357 (M^+)

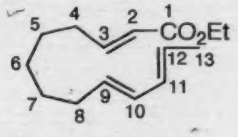


(2*E*,8*E*,10*E*)-Ethyl dodeca-2,8,10-trienoate (50a).[131]

A 50 mL round bottom flask with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this flask was placed Wittig reagent **38** (0.0144 mol, 5.02 g) and CH_2Cl_2 (25 mL). This was stirred at room temperature until the Wittig reagent **38** was completely dissolved. Then, diene aldehyde **50** (~0.012 mol, 1.83 g), was added and the reaction mixture was stirred overnight at room temperature. The solvent was removed *in vacuo*. Then, the product was was purified by flash chromatography (SiO_2, gradient elution, 5% Et_2O:pentane, 10% Et_2O:pentane). This provided the all *E*-dodecatriene ester **50a** ($2.13x10^{-3}$ mol, 473 mg) as a volatile oil in a yield of 18%. The *Z* isomer was readily separated under these conditions and was not utilized.

R_f 0.32 (10% EtOAc:hexanes); IR (CH_2Cl_2 cast) 3015, 2930, 2856, 1722, 1654, 1447 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 6.95 (dt, 1H, J = 15.7, 6.9 Hz, H3), 6.03-5.97 (m, 2H, H9,H10), 5.80 (dt, 1H, J = 15.7, 1.7 Hz, H2), 5.60-5.42 (m, 2H, H8,H11), 4.18 (q, 2H, J = 7.1 Hz, OCH_2CH_3), 2.19 (dq, 2H, J = 1.3, 7.0 Hz, H4), 2.06 (q, 2H, J = 7.1 Hz, H7), 1.73 (d, 3H, J = 6.5 Hz, H12), 1.50-1.37 (m, 4H,

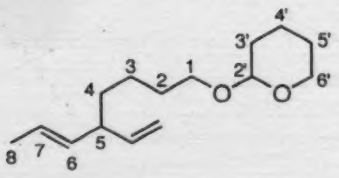
H5,H6), 1.29 (t, 3H, J = 7.1 Hz, $OCH_2C\underline{H}_3$); HRMS (EI) calcd for $C_{14}H_{22}O_2$ 222.1620, found 222.1615 (M^+)



(2*E*,9*E*,11*E*)-Ethyl trideca-2,9,11-trienoate (51a).

The procedure followed was the same as that used to prepare (2*E*,8*E*,10*E*)-ethyl dodeca-2,8,10-trienoate (**50a**). For this preparation the amounts of reagents used were undecadienal **51** ($9.98x10^{-3}$ mol, 1.65 g), Wittig reagent **38** ($1.12x10^{-2}$ mol, 4.17 g) and CH_2Cl_2 (80 mL). The product was purified by flash chromatography (SiO_2, gradient elution, 5% Et_2O:pentane, 10% Et_2O:pentane). This provided tridecatrienoate **51a** ($8.48x10^{-3}$ mol, 2.0 g) as a volatile oil in a yield of 85%.

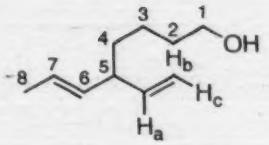
R_f 0.45 (10% EtOAc:hexanes); IR ($CHCl_3$ cast) 2929, 2856, 1719, 1653, 1559 cm^{-1}; ^1H NMR ($CDCl_3$, MHz) δ 6.96 (dt, 1H, J = 15.6, 6.9 Hz, H3), 6.05-5.97 (m, 2H, H9,H10), 5.81 (dt, 1H, J = 15.6, 1.7 Hz, H2), 5.61-5.51 (m, 2H, H8,H11), 4.19 (q, 2H, J = 7.1 Hz, $OC\underline{H}_2CH_3$), 2.19 (dq, 2H, J = 1.7, 7.1 Hz, H4), 2.05 (q, 2H, J = 7.1 Hz, H8), 1.74 (m, 3H), 1.49-1.44 (m, 2H), 1.42-1.31 (m, 4H), 1.29 (t, 3H, J = 7.1 Hz, $OCH_2C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, MHz) δ 166.7, 149.2, 131.6, 131.5, 130.3, 126.8, 121.2, 60.0, 32.3, 32.0, 29.1, 28.5, 27.8, 17.9, 14.2; HRMS (EI) calcd for $C_{15}H_{24}O_2$ 236.1776, found 236.1775 (M^+)



(*E*)-2-(5-Vinyloct-6-enyloxy)tetrahydro-2*H*-pyran (52).

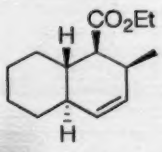
This compound was isolated from the preparation of 2-((6*E*,8*E*)-deca-6,8-dienyloxy)tetrahydro-2*H*-pyran (**48**). The data presented is for the mixture of 2 diastereomers.

R_f 0.64 (10% EtOAc:hexanes, SiO_2) 0.2 (10% Et_2O:pentane, $AgNO_3$•SiO_2); IR (CH_2Cl_2 cast) 3077, 2939, 1653, 1636 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.85 (ddt, 1H, *J* = 17.2, 10.3, 6.8 Hz), 5.70 (ddd, 1H, *J* = 7.4, 10.3, 17.2 Hz), 5.42 (ddq, 1H, *J* = 0.83, 6.3, 15.2 Hz), 5.32 (ddq, 1H, *J* = 1.3, 7.6, 15.2 Hz), 5.10 (dq, 1H, *J* = 1.6, 17.2 Hz), 5.04 (ddt, 1H, *J* = 10.3, 2.0, 1.2 Hz), 4.98 (ddd, 1H, *J* = 1.3, 1.9, 12.6 Hz), 4.95 (ddd, 1H, *J* = 1.2, 1.9, 5.8 Hz), 4.60 (dd, 1H, *J* = 3.0, 4.3 Hz), 4.57 (dd, 1H, *J* = 2.9, 4.5 Hz), 3.90-3.84 (m, 2H), 3.79 (dt, 1H, *J* = 9.6, 7.0, Hz), 3.72 (dt, 1H, *J* = 9.6, 7.0 Hz), 3.53-3.44 (m, 3H), 3.38 (dt, 1H, *J* = 9.5, 6.8 Hz), 2.67-2.60 (m, 1H), 2.37 (qt, 1H, *J* = 1.4, 6.9 Hz), 1.87-1.80 (m, 2H), 1.75-1.69 (m, 2H), 1.68-1.66 (m, 6H), 1.62-1.50 (m, 10H), 1.42-1.22 (m, 12H); HRMS (EI) calcd for $C_{15}H_{26}O_2$ 238.1933, found 238.1932 (M^+)



(*E*)-5-Vinyloct-6-en-1-ol (53).

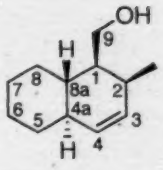
The procedure used to prepare this compound was the same as that used for (6*E*,8*E*)-deca-6,8-dien-1-ol (**48a**). For this reaction, (*E*)-2-(5-vinyloct-6-enyloxy)tetrahydro-2*H*-pyran (**52**) (2.52x10^{-3} mol, 0.6 g), CH_3OH (20 mL) and *p*-TsOH (8.99x10-5 mol, 17.1 mg) were used. After purification this provided (*E*)-5-vinyloct-6-en-1-ol (**53**) (2.5x10-3 mol, 385 mg) as a clear oil in quantitative yield. R_f 0.12 (20% EtOAc:hexanes); IR ($CHCl_3$ cast) 3328, 3078, 3021, 1637, 1452 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.70 (ddd, 1H, *J* = 7.5, 10.1, 17.1 Hz, H_a), 5.42 (ddq, 1H, *J* = 0.99, 6.4, 15.3 Hz, H7), 5.31 (ddq, 1H, *J* = 1.5, 7.5, 15.3 Hz, H6), 4.99-4.94 (m, 2H, H_b, H_c), 3.63 (t, 2H, *J* = 6.6 Hz, H1), 2.63 (dddd, 1H, *J* = 6.9, 7.0, 7.1, 7.4 Hz, H5), 1.67 (ddd, 3H, *J* = 0.77, 1.5, 6.3 Hz, H8), 1.58-1.53 (m, 2H, H2), 1.45 (br s, 1H, OH), 1.42-1.32 (m, 4H, H3,H4); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 142.1, 133.7, 124.8, 113.3, 62.9, 46.7, 34.6, 32.5, 23.1, 17.9; HRMS (EI) calcd for $C_{10}H_{18}0$ 154.1358, found 154.13536 (M^+)



(±)-(1*S*,2*S*,4a*R*,8a*S*)-Ethyl 2-methyl-1,2,4a,5,6,7,8,8a-octahydronapthalene-1-carboxylate (50b).[40, 67, 78]

A 25 mL round bottom flask with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this flask was placed ethyl dodecatrienoate **50a** (5.23×10^{-4} mol, 116 mg) and toluene (1 mL). The solution was cooled to 0 °C and then ethylaluminum dichloride (5.24×10^{-4} mol, 291 μL, of a 1.8 M solution in toluene) was added slowly. The reaction mixture was stirred at room temperature for 5 hours. To the reaction mixture was added HCl (2 mL of a 1 M solution) and the solution was stirred for 10 minutes. Then, the reaction mixture was transferred to a 30 mL separatory funnel and extracted with Et_2O (3x5 mL). The ethereal extracts were dried over Na_2SO_4 and the solvent was removed *in vacuo*. The products were purified using flash chromatography (SiO_2, isocratic elution, 3% Et_2O:pentane). This provided the decalin-ethylester **50b** (1.57×10^{-4} mol, 35 mg) as a volatile oil in a yield of 30%. This was used directly in the next reaction (with $LiAlH_4$) to minimize loss of material by evaporation.

R_f 0.48 (5% EtOAc:hexanes)

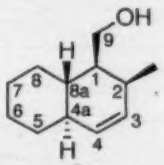


(±)-((1*S*,2*S*,4a*R*,8a*S*)-2-methyl-1,2,4a,5,6,7,8,8a-octahydronapthalen-1-yl)methanol (55).[132]

A 25 mL round bottom flask with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this flask was placed decalin-ethyl ester **50b** (5.53×10^{-4} mol, 123 mg) and THF (10 mL). This mixture was cooled to 0 °C. Then, $LiAlH_4$ (0.55 mL of a 1M solution in THF) was added slowly. The mixture was stirred for one hour during which time the reaction mixture was allowed to warm to room temperature. The reaction mixture was cooled to 0 °C and quenched by the sequential addition of water (2 mL), NaOH (2 mL of 3 M solution) and water (3 mL). This solution was stirred vigorously at room temperature for one hour. To the stirred solution was added $MgSO_4$ (~0.75 g). The solid was removed by vacuum filtration and the solvent was removed *in vacuo*. The resultant alcohol was purified using flash chromatography (SiO_2, isocratic elution, 10% EtOAc:hexanes). This provided decalin alcohol **55** (4.98×10^{-4} mol, 90 mg) as a white, crystalline solid in a yield of 90%.

R_f 0.64 (30% EtOAc:hexanes); IR (CH_2Cl_2 cast) 3346, 3008, 2921, 2853, 1652 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.60 (ddd, 1H, J = 2.5, 4.5, 9.5 Hz, H3), 5.38 (d, 1H, J = 9.5 Hz, H4), 3.85 (dd, 1H, J = 5.5, 11.0 Hz, H9), 3.55 (dd, 1H, J = 9.2, 11.0 Hz, H9), 2.46-2.40 (m, 1H, H2), 1.82-1.68 (m, 6H, H1,H4a,H6,H7,H8), 1.35-

1.21 (m, 2H, H5), 1.15-0.98 (m, 3H, H7,H8,H8a), 0.94 (d, 3H, J = 7.0 Hz, C$\underline{H}_3$2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 132.3 (C3), 131.2 (C4), 63.1 (C9), 44.2 (C1), 43.6 (C4a), 37.7 (C8a), 33.2 (C7,8), 31.5 (C2), 29.3 (C7,8), 26.8 (C6), 26.5 (C5), 15.5 (C$\underline{H}_3$2); HRMS (EI) calcd for $C_{12}H_{20}O$ 180.1514, found 180.1512 (M^+)

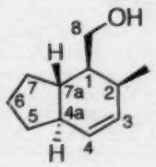


(±)-((1*S*,2*S*,4a*R*,8a*S*)-2-methyl-1,2,4a,5,6,7,8,8a-octahydronapthalen-1-yl)methanol (55).

Into a 10 mL round bottom flask with a magnetic stirrer bar was placed IBX ($3.94x10^{-4}$ mol, 110 mg) and DMSO (5 mL). The flask was fitted with a rubber septum and stirred at room temperature under Ar until the IBX was completely dissolved (~30 minutes). Then, dodecatrienol **30** ($2.72x10^{-4}$ mol, 49.0 mg) was added in one portion and the mixture was stirred overnight at room temperature. The reaction flask was then cooled to 0 °C and water (3 mL) was added. The mixture was stirred for 20 minutes at room temperature and then vacuum filtration was used to remove the white solid. The solid was rinsed with Et_2O (3x10 mL) and the entire filtrate was transferred to a 60 mL separatory funnel. The aqueous layer was removed, the organic extracts were washed with brine (15 mL) and dried over Na_2SO_4. The solid Na_2SO_4 was removed by gravity filtration into a flame dried, 50 mL round bottom flask equipped with a magnetic stirrer bar. The flask was fitted with a rubber septum and cooled to 0 °C with

stirring under Ar. Then, $NaBH_4$ ($2.72x10^{-4}$ mol, 10 mg) was added in one portion to the flask. The mixture was stirred at 0 °C and monitored by TLC. Once the reaction was judged to be complete, it was quenched by the addition of water (2 mL dropwise) followed by HCl (1 M solution dropwise) until H_2 evolution ceased. Then the mixture was transferred to a 60 mL separatory funnel and washed sequentially with water (15 mL), brine (15 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo*. The product was purified using flash chromatography (SiO_2, isocratic elution, 10% EtOAc:hexanes). This provided decalin alcohol **55** ($2.18x10^{-4}$ mol, 39 mg) as a white, crystalline solid with a yield of 80% over two steps.

R_f 0.64 (30% EtOAc:hexanes); IR (CH_2Cl_2 cast) 3346, 3008, 2921, 2853, 1652 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.60 (ddd, 1H, J = 2.5, 4.5, 9.5 Hz, H3), 5.38 (d, 1H, J = 9.5 Hz, H4), 3.85 (dd, 1H, J = 5.5, 11.0 Hz, H9), 3.55 (dd, 1H, J = 9.2, 11.0 Hz, H9), 2.46-2.40 (m, 1H, H2), 1.82-1.68 (m, 6H, H1,H4a,H6,H7,H8), 1.35-1.21 (m, 2H, H5), 1.15-0.98 (m, 3H, H7,H8,H8a), 0.94 (d, 3H, J = 7.0 Hz, C$\underline{H}_3$2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 132.3 (C3), 131.2 (C4), 63.1 (C9), 44.2 (C1), 43.6 (C4a), 37.7 (C8a), 33.2 (C7,8), 31.5 (C2), 29.3 (C7,8), 26.8 (C6), 26.5 (C5), 15.5 (C$\underline{H}_3$2); HRMS (EI) calcd for $C_{12}H_{20}O$ 180.1514, found 180.1512 (M^+)



(±)-((3a*S*,4*S*,5*S*,7a*R*)-5-Methyl-2,3,3a,4,5,6,7a-hexahydro-1*H*-inden-4-yl)methanol (56).

The procedure used to prepare indene alcohol **56** was the same as that used for decalin alcohol **55**. The amounts of reagents used were undecatrienol **29** (2.26×10^{-4} mol, 47 mg), IBX (4.08×10^{-4} mol, 115 mg) and DMSO (5 mL). The intermediate aldehyde was reduced with $NaBH_4$ (2.26×10^{-4} mol, 9 mg) in Et_2O (30 mL). The product was purified using flash chromatography (SiO_2, isocratic elution, 20% EtOAc:hexanes). This provided indene alcohol **56** (1.70×10^{-4} mol, 28 mg) as a white solid in a yield of 75%.

R_f 0.60 (30% EtOAc:hexanes); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.75 (d, 1H, J = 9.8 Hz, H4), 5.53 (ddd, 1H, J = 3.2, 4.0, 9.8 Hz, H3), 3.72 (dd, 1H, J = 5.6, 10.7 Hz, H8), 3.59 (dd, 1H, J = 9.5, 10.7 Hz, H8), 2.56-2.50 (m, 1H, H2), 1.90-1.66 (m, 5H), 1.35-1.05 (m, 4H), 0.93 (d, 3H, J = 7.2 Hz, C$\underline{H}_3$2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 133.6, 128.5, 64.1, 46.5, 45.1, 41.4, 32.5, 28.9, 27.6, 22.7, 15.8

General procedure for the ADH catalyzed oxidations

Into a 25 mL Erlenmeyer flask was placed glycine sodium hydroxide buffer (10 mL at pH 8.76 for YADH and HLADH, pH 8.90 for SDH), enzyme (5 mg) and

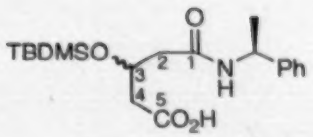
NAD^+ (7.9 mg). This solution was placed on an orbital shaker for one hour at room temperature. Then the substrate (5 mg in 200 μL of acetone or neat) was added in one portion to the reaction flask. The flask was sealed with parafilm and placed on an orbital shaker for 3 to 5 days. Then the reaction mixture was transferred to a test tube and placed in a bench top centrifuge for two hours. The supernatant was decanted into a 1 dram vial and extracted with hexane (4x1 mL). The solvent was removed *in vacuo* and the residue was taken up in 95% ethanol and reduced with $NaBH_4$ at room temperature overnight. The procedure used to isolate the products of the reaction was identical to the procedure used for the isolation of decalin alcohol **55**. The solid residue was dissolved in distilled CH_2Cl_2 (1 mL) and placed into a GC-MS vial and submitted to GC-MS analysis. The retention times in minutes for the products were as follows:

Decatrienol **28** diastereomers 36.3, 37.5, 40.4, 42.1 and 43.9 minutes for starting material, respectively

Undecatrienol **29** diastereomers 52.2, 53.8, 57.1, 58.0 and 55.8 minutes for starting material, respectively

Dodectrienol **30** diastereomers *exo* (62.5, 63.2), endo (64.9, 65.7) and 67.5 minutes for starting material, respectively

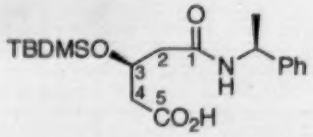
Tridecatrienol **31** diastereomers 55.4, 57.0, 61.9, 62.1 and 60.6 minutes for starting material, respectively



(±)-3-(*tert*-Butyldimethylsilyloxy)-5-oxo-5-(1-phenylethylamino)pentanoic-acid (66 and 67).[90]

A 3 neck, 100 mL round bottom flask with a magnetic stirrer bar was flame dried under Ar. Into this flask was placed 3-(*tert*-butyldimethylsilyloxy)glutaric anhydride (**64**) (5.98×10^{-3} mol, 1.46 g) and toluene (30 mL). This mixture was stirred at room temperature until the anhydride was completely dissolved. Then the reaction flask was cooled to -78 °C (dry ice:acetone). After 5 minutes NEt_3 (5.98×10^{-3} mol, 0.83 mL) and (*S*)-(-)-α-methylbenzylamine **60** (6.58×10^{-3} mol, 0.84 mL) were added sequentially to the reaction flask. This mixture was stirred at -78 °C for 4.5 hours and then at room temperature overnight. The reaction mixture was transferred to 125 mL separatory funnel and diluted with EtOAc (15 mL) and THF (7 mL). The organic layer was washed sequentially with $KHSO_4$ (25 mL of 5% aqueous solution), brine (2x25 mL) and then dried over Na_2SO_4. The solvent was removed *in vacuo* to provide the amide acids **66** and **67** (5.6×10^{-3} mol, 2.05 g) as a white solid in a yield of 94% and *syn*:*anti* ratio of 85:15. The *syn*-amide acid **66** was isolated by washing with ice cold Et_2O to provide this compound pure as white flakes. The data recorded is for the mixture of diastereomers **66** and **67**.

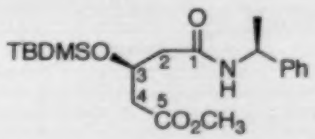
R_f 0.51 (10% CH_3OH:CH_2Cl_2); IR ($CHCl_3$ cast) 3400-2500, 3291, 3064, 3031, 2954, 2929, 2895, 2856, 1713, 1640, 1545 cm^{-1}; ^1H NMR (DMSO, 500 MHz) δ 8.31 (d, 1H, J = 7.5 Hz, NH major), 8.25 (d, 1H, J = 8.8 Hz, NH minor), 7.30-7.26 (m, 4H, ArH), 7.21-7.17 (m, 1H, ArH), 4.93-4.86 (m, 1H, NHCH(CH_3)), 4.46-4.40 (m, 1H, H3), 2.45-2.23 (m, 4H, H2,H4), 1.34 (d, 3H, J = 8.8 Hz, NHCH(CH_3) minor), 1.31 (d, 3H, J = 7.5 Hz, NHCH(CH_3) major), 0.81 (s, 9H, SiC(CH_3)$_3$ major), 0.75 (s, 9H, SiC(CH_3)$_3$ minor), 0.03 (s, 3H, SiCH_3), 0.02 (s, 3H, SiCH_3); ^{13}C NMR (DMSO, 125 MHz) δ 172.1, 168.5 (major), 168.3 (minor), 144.7 (major), 144.2 (minor), 128.03 (major), 127.99 (minor), 126.44 (minor), 126.37 (major), 126.0 (minor), 125.7 (major), 66.8 (major), 66.7 (minor), 47.5, 43.5, 42.4, 25.6, 22.4 (minor), 22.3 (major), 17.6, -4.9, -5.1; HRMS (EI) calcd for $C_{19}H_{31}NO_4Si$ 365.2022, found 365.2019 (M^+)



(*S*)-3-(*tert*-Butyldimethylsilyloxy)-5-oxo-5-((*S*)-1-phenylethylamino)-pentanoic acid (66).[90]

R_f 0.44 (10% CH_3OH:CH_2Cl_2); $[\alpha]_D^{25}$ -65.8° (*c* 0.38, CH_3OH); IR (CH_3OH cast) 3321, 3096, 3030, 2929, 2857, 1696, 1621, 1561, 1494 cm^{-1}; ^1H NMR (DMSO, 500 MHz) δ 8.31 (d, 1H, J = 7.7 Hz, NH), 7.31-7.27 (m, 4H, *o,m* ArH), 7.21-7.18 (m, 1H, *p* ArH), 4.89 (dq, 1H, J = 7.1, 7.7 Hz, NH(CH)CH_3), 4.44 (dddd, 1H, J = 4.8, 4.9, 7.7, 7.7 Hz, H3), 3.30 (br s, 1H, OH), 2.376 (dd, 1H, J = 4.8, 15.1 Hz,

H4'), 2.364 (dd, 1H, J = 7.7, 14.0 Hz, H2), 2.28 (dd, 1H, J = 7.7, 15.1 Hz, H4), 2.25 (dd, 1H, J = 4.9, 14.0 Hz, H2'), 1.31 (d, 3H, J = 7.1 Hz, NH(CH)C$\underline{H}_3$), 0.82 (s, 9H, SiC(C$\underline{H}_3$)$_3$), 0.03 (s, 3H, SiC$\underline{H}_3$), 0.02 (s, 3H, SiC$\underline{H}_3$); ^{13}C NMR (DMSO, 125 MHz) δ 172.1 (C5), 168.5 (C1), 144.7 (C4°), 128.0 (C*ortho*), 126.4 (C*para*), 125.7 (C*meta*), 66.8 (C3), 47.5 (NH(C$\underline{H}$)CH$_3$), 43.5 (C2), 42.4 (C4), 38.9 (SiC(C$\underline{H}_3$)$_3$), 22.3 (NH(CH)C$\underline{H}_3$), 17.6 (SiC(CH$_3$)$_3$), -4.9 (SiC$\underline{H}_3$), -5.1 (SiC$\underline{H}_3$); HRMS (EI) calcd for $C_{19}H_{31}NO_4Si$ 365.2022, found 365.2023 (M$^+$)

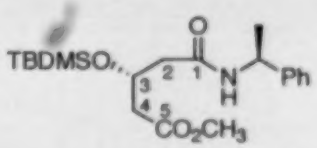


(*S*)-Methyl-3-(*tert*-butyldimethylsilyloxy)-5-oxo-5-((*S*)-1-phenylethylamino)-pentanoate (68).[90]

A 100 mL round bottom flask with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this flask were placed amide acids **66** and **67** (5.6x10^{-3} mol, 2.05 g) and DMF (30 mL). The mixture was stirred at room temperature until the acid had completely dissolved. Then methyl iodide (0.011 mol, 0.7 mL) and $KHCO_3$ (0.011 mol, 1.12 g) were added. The reaction mixture was stirred for two days at room temperature under Ar. The reaction mixture was diluted with water (15 mL) and transferred into a 125 mL separatory funnel by rinsing with EtOAc (30 mL). The organic layer was washed sequentially with water (3x10 mL), brine (10 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* to give a viscous yellow oil. The diastereomers were

separated by flash chromatography (SiO_2, isocratic elution, 40% EtOAc:hexanes). This provided the *syn*-methyl ester **68** ($3.75x10^{-3}$ mol, 1.43 g) and the *anti*-methyl ester **69** ($6.62x10^{-4}$ mol, 251 mg) in yields of 67% and 12% respectively. The *syn*-methyl ester **68** solidified upon standing whereas the *anti*-methyl ester **69** remained a viscous, oil.

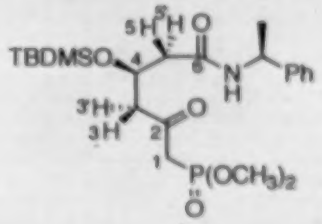
R_f 0.63 (40% EtOAc:hexanes); IR ($CHCl_3$ cast) 3292, 3064, 3030, 2953, 2929, 2895, 2856, 1741, 1641, 1543, 1495 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.36-7.28 (m, 4H, ArH), 7.25-7.21 (m, 1H, ArH), 6.55 (d, 1H, J = 7.5 Hz, NH), 5.10 (dq, 1H, J = 7.3, 7.5 Hz, NHCH(CH_3)), 4.50 (dddd, 1H, J = 5.4, 5.7, 5.8, 6.0 Hz, H3), 3.65 (s, 3H, OCH_3), 2.54 (dd, 1H, J = 5.4, 14.7 Hz, H4), 2.46 (dd, 2H, J = 3.0, 6.0 Hz, H2), 2.39 (dd, 1H, J = 5.0, 14.7 Hz, H4), 1.47 (d, 3H, J = 7.5 Hz, NHCH(CH_3)), 0.86 (s, 9H, $SiC(CH_3)_3$), 0.11 (s, 3H, $SiCH_3$), 0.08 (s, 3H, $SiCH_3$); HRMS (EI) calcd for $C_{20}H_{33}NO_4Si$ 379.2179, found 379.2179 (M^+)



(*R*)-Methyl 3-(*tert*-butyldimethylsilyloxy)-5-oxo-5-((*S*)-1-phenylethylamino)-pentanoate (69).[90]

R_f 0.53 (40% EtOAc:hexanes); IR ($CHCl_3$ cast) 3293, 3064, 3030, 2953, 2929, 2895, 2856, 1741, 1640, 1543, 1495 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.36-7.29 (m, 4H, ArH), 7.28-7.22 (m, 1H, ArH), 6.53 (d, 1H, J = 6.5 Hz, NH), 5.12 (dq, 1H, J = 6.5, 7.3 Hz, NHCH(CH_3)), 4.52 (dddd, 1H, J = 5.0, 5.2, 5.5, 6.0 Hz, H3),

3.69 (s, 3H, OCH_3), 2.59 (d, 2H, J = 5.7 Hz, H2), 2.55 (dd, 1H, J = 5.1, 14.9 Hz, H4), 2.40 (dd, 1H, J = 4.8, 14.9 Hz, H4), 1.55 (d, 3H, J = 6.5 Hz, $NHCH(CH_3)$), 0.80 (s, 9H, $SiC(CH_3)_3$), 0.07 (s, 3H, $SiCH_3$), 0.03 (s, 3H, $SiCH_3$); HRMS (EI) calcd for $C_{20}H_{33}NO_4Si$ 379.2179, found 379.2178 (M^+)

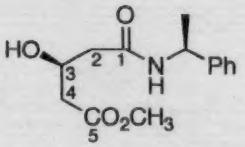


Dimethyl (*R*)-4-(*tert*-butyldimethylsilyloxy)-2,6-dioxo-6-((*S*)-1-phenylethylamino)hexylphosphonate (70).[100]

A 10 mL round bottom flask with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this flask was placed *O,O*-dimethyl methylphosphonate ($3.22x10^{-4}$ mol, 40 μL) and dry THF (3.5 mL). The reaction mixture was cooled to -78 °C (dry ice:acetone) at which point n-BuLi (0.18 mL of 1.6 M solution in THF) was added slowly via a 1 mL syringe. The reaction mixture was stirred at -78 °C for 20 minutes. Then a solution of *syn*-methyl ester **68** ($2.68x10^{-4}$ mol, 101.7 mg, in 1.5 mL THF) was added (dropwise, 3x0.5 mL) over 25 minutes to the reaction flask. The solution was stirred for 5 minutes, then the reaction was quenched by the addition of saturated NH_4Cl (0.5 mL). The reaction mixture was allowed to warm up to room temperature over 1 hour, with stirring under Ar. The reaction mixture was transferred to a 30 mL separatory funnel with EtOAc (10 mL). The organic layer was washed with water (2x5 mL)

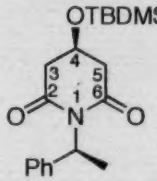
then brine (2x5 mL). The combined aqueous washes were extracted with EtOAc (2x10 mL) and all organic extracts were combined and dried over Na_2SO_4. The solvent was removed *in vacuo* and the product was purified using flash chromatography (SiO_2, isocratic 100% EtOAc). This provided the amide phosphonate **70** ($7.65x10^{-4}$ mol, 36.1 mg) as a viscous oil in a 30% yield.

R_f 0.20 (100% EtOAc); $[\alpha]_D^{25}$ -29.1° (*c* 0.93, $CHCl_3$); IR ($CHCl_3$ cast) 3291, 2956, 2928, 1713, 1646, 1542 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.32-7.29 (m, 4H, *o,m* ArH), 7.24-7.22 (m, 1H, *p* ArH), 6.56 (d, 1H, *J* = 8.0 Hz, NH), 5.09 (dq, 1H, *J* = 8.0, 14.1 Hz, NHCH(CH_3)), 4.47 (dddd, 1H, *J* = 5.3, 5.4, 5.4, 5.5 Hz, H4), 3.71 (d, 3H, *J(H,P)* = 9.3 Hz, OCH_3), 3.68 (d, 3H, *J(H,P)* = 9.3 Hz, OCH_3), 3.03 (dd, 1H, *J(H,H)* = 13.9 Hz, *J(H,P)* = 22.5 Hz, H1), 2.97 (dd, 1H, *J(H,H)* = 13.9 Hz, *J(H,P)* = 22.5 Hz, H1), 2.76 (dd, 1H, *J* = 6.3, 16.7 Hz, H3), 2.67 (dd, 1H, *J* = 5.7, 16.7 Hz, H3'), 2.47 (dd, 1H, *J* = 5.2, 14.7 Hz, H5), 2.32 (dd, 1H, *J* = 4.6, 14.7 Hz, H5'), 1.44 (d, 3H, *J* = 7.0 Hz, NHCH(CH_3)), 0.84 (s, 9H, SiC(CH_3)$_3$), -0.09 (s, 3H, Si(CH_3)), -0.06 (s, 3H, Si(CH_3)); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 200.0 (d, *J(C,P)* = 6.3 Hz, C2), 169.1 (C6), 143.5 (C4°Ar), 128.6 (C*ortho*), 127.2 (C*meta*), 126.1 (C*para*), 65.9 (C4), 52.95 (d, *J(C,P)* = 6.2 Hz, OCH_3), 52.88 (d, *J(C,P)* = 6.2 Hz, OCH_3), 49.6 (C3), 48.7 (NHCH(CH_3), 43.5 (C5), 42.4 (d, *J(C,P)* = 127.8 Hz, C1). 25.7 (SiC(CH_3)$_3$), 22.0 (NHCH(CH_3), 17.8 (SiC(CH_3)$_3$), -4.97 (SiCH_3), -5.01 (SiCH_3); ^{31}P NMR ($CDCl_3$, 162 MHz) δ 23.2



Methyl 3(*S*)-hydroxy-5-oxo-5-((*S*)-1-phenylethylamino)pentanoate (71).

R_f 0.4 (100% EtOAc); $[\alpha]_D^{25}$ -11.3 (*c* 0.88, $CHCl_3$); IR ($CHCl_3$ cast) 3301, 3064, 2974, 2953, 2930, 1736, 1643, 1544, 1495 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.36-7.30 (m, 4H, *o,m* ArH), 7.28-7.26 (m, 1H, *p* ArH), 6.32 (d, 1H, *J* = 7.1 Hz, NH), 5.13 (dq, 1H, *J* = 7.1, 7.1 Hz, NH(CH)CH_3), 4.40 (dddd, 1H, *J* = 3.7, 5.1, 7.9, 7.9 Hz, H3), 3.71 (s, 3H, OCH_3), 2.55 (dd, 1H, *J* = 7.9, 16.3 Hz, H4), 2.50 (dd, 1H, *J* = 5.1, 16.3 Hz, H4'), 2.44 (dd, 1H, *J* = 3.7, 15.2 Hz, H2'), 2.40 (dd, 1H, *J* = 7.9, 15.2 Hz, H2), 1.50 (d, 3H, *J* = 7.1 Hz, NH(CH)CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.4 (C5), 170.1 (C1), 142.9 (C4°), 128.6 (C*ortho*), 127.3 (C*para*), 126.0 (C*meta*), 65.2 (C3), 51.8 (OCH_3), 48.7 (NH(CH)CH_3), 41.8 (C2), 40.4 (C4), 21.8 (NH(CH)CH_3); HRMS (EI) calcd for $C_{14}H_{19}O_4N$ 265.1314, found 265.1318 (M^+)

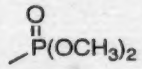


(*S*)-4-(*tert*-Butyldimethylsilyloxy)-1-(1-phenylethyl)piperidine-2,6-dione (72).[93]

A 50 mL round bottom flask with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this flask was placed *syn*-methyl ester **68**

($2.51x10^{-4}$ mol, 95.1 mg), $NH(CH_3)OCH_3$•HCl ($3.88x10^{-4}$ mol, 47.8 mg) and THF (5 mL). The reaction mixture was cooled to -15 °C (dry ice:ethylene glycol) and then *i*PrMgCl ($8.0x10^{-4}$ mol, 0.4 mL of 2 M solution in THF) was added. The reaction was stirred at -15 °C for 3 hours. The reaction was then quenched via addition of NH_4Cl (5 mL). The solution was transferred to a 60 mL separatory funnel and extracted with EtOAc (3x10 mL). The combined organic extracts were washed sequentially with water (15 mL), brine (15 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the product was purified using flash chromatography (SiO_2, isocratic elution, 20% EtOAc:hexanes). This provided the glutamide **72** ($1.30x10^{-4}$ mol, 45.1 mg) as a solid in a yield of 46%.

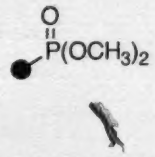
R_f 0.80 (50% EtOAc:hexanes); $[\alpha]_D^{25}$ -109° (*c* 0.35, $CHCl_3$); IR ($CHCl_3$ cast) 2955, 2930, 2857, 1731, 1682, 1498 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.33-7.30 (m, 2H, *o* ArH), 7.27-7.24 (m, 2H, *m* ArH), 7.19-7.16 (m, 1H, *p* ArH), 6.07 (q, 1H, *J* = 7.0 Hz, NCH(CH_3)), 4.28-4.25 (m, 1H, H4), 2.80-2.67 (m, 4H, H3,H5), 1.74 (d, 3H, *J* = 7.0 Hz, NCH(CH_3)), 0.84 (s, 9H, SiC(CH_3)$_3$), 0.06 (s, 3H, SiCH_3), 0.04 (s, 3H, SiCH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.5, 170.3, 140.7, 128.0, 126.7, 126.6, 62.5, 49.4, 42.22, 42.19, 25.5, 16.3, -4.99, -5.01; HRMS (+ES) calcd for $C_{19}H_{29}NO_3SiNa$ 370.1814, found 370.1816 (M^++Na)



_O,O_-Dimethyl methylphosphonate (74a).[94, 133]

A 25 mL round bottom flask with a magnetic stirrer bar was fitted with a condenser and flame dried under Ar. Into this flask was placed trimethyl phosphite **73** (7.63×10^{-3} mol, 0.9 mL) and methyl iodide (7.63×10^{-3} mol, 0.48 mL). The mixture was heated at 40 °C with an oil bath overnight. The excess methyl iodide was removed *in vacuo* to provide *O,O*-dimethyl methylphosphonate (**74a**) (5.44×10^{-3} mol, 674.4 mg) as an oil in a yield of 71%.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.70 (d, 6H, *J(H,P)* = 10.8 Hz, $OC\underline{H}_3$), 1.44 (d, 3H, *J(H,P)* = 17.6 Hz, $C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 52.2 (d, *J(C,P)* = 6.3 Hz, $O\underline{C}H_3$), 9.85 (d, *J(C,P)* = 144.5 Hz, $\underline{C}H_3$); ^{31}P NMR ($CDCl_3$, 162 MHz) δ 34.3

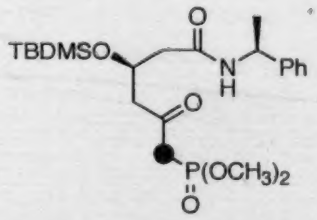


_O,O_-Dimethyl-$^{13/14}$C-methylphosphonate (74).[94, 134]

The ^{14}C-methyl iodide (250 μCi, specific activity 6.1 mCi mmol^{-1}) was transferred to the reaction flask containing trimethyl phosphite **73** (3.5×10^{-3} mol, 0.41 mL) by dilution with ^{13}C-methyl iodide (3.5×10^{-3} mol, 0.22 mL). The detailed apparatus and procedure are given in appendix A. The reaction conditions were the same as that used for the preparation of unlabeled *O,O*-dimethyl methylphosphonate (**74a**). This provided *O,O*-dimethyl-$^{13/14}$C-methylphosphonate (**74**) (3.47×10^{-3} mol,

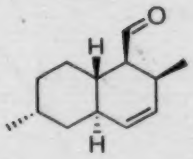
0.43 g, total activity 5.65 μCi) with a quantitative yield and incorporation of carbon 13 and 14.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.72 (d, 6H, *J(H,P)* = 11.0 Hz, $OC\underline{H}_3$), 1.45 (d, 3H, *J(H,P)* = 17.5 Hz, $^{13/14}C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 52.2 (d, *J(C,P)* = 6.6 Hz, $O\underline{C}H_3$), 9.88 (d, *J(C,P)* = 149.9 Hz, $^{13/14}C\underline{H}_3$); ^{31}P NMR ($CDCl_3$, 162 MHz) δ 34.2



[$^{13/14}$C]1-Dimethyl (*R*)-4-(*tert*-butyldimethylsilyloxy)-2,6-dioxo-6-((*S*)-1-phenylethylamino)hexylphosphonate (76).

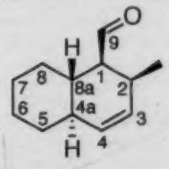
The procedure followed for the preparation of the $^{13/14}$C-amide phosphonate **76** was identical as the one used for unlabeled amide phosphonate **70**. For this preparation the amount of *syn*-methyl ester **68** ($1.09x10^{-3}$ mol, 0.41 g) and labeled dimethyl methylphosphonate **74** ($1.31x10^{-3}$ mol, 0.16 g, total activity 2.12 μCi) were divided into to four separate reactions. When the reactions were completed, the crude material was combined for purification. In this way the labeled $^{13/14}$C-amide phosphonate **76** ($2.68x10^{-4}$ mol, 126.3 mg, total activity 0.55 μCi) was obtained as a viscous, yellow oil in a yield of 25%. Spectral data matched those obtained for amide phosphonate **70**.



(1*S*,2*S*,4a*R*,6*R*,8a*S*)-2,6-Dimethyl-1,2,4a,5,6,7,8,8a-octahydronapthalene-1-carbaldehyde (78).

The procedure used to prepare compound **78** was the same as that used to prepare decalin aldehyde **79**. The amounts of reagents used were 6-Me-decalin alcohol **84** (2.98×10^{-4} mol, 58 mg), IBX (3.58×10^{-4} mol, 100 mg) and DMSO (10 mL). The product was purified using flash chromatography (SiO_2, isocratic elution, 3% Et_2O:pentane). This provided the 6-Me-decalin aldehyde **78** (2.92×10^{-4} mol, 56 mg) as a volatile oil in a yield of 98%. This was used directly in the next reaction (HEW) to minimize loss due to evaporation.

R_f 0.75 (10% EtOAc:hexanes)

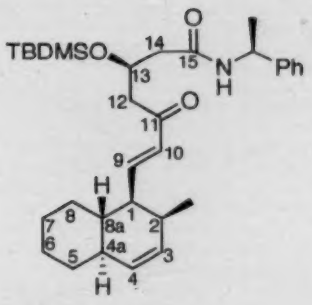


(±)-(1*S*,2*S*,4a*R*,8a*S*)-2-Methyl-1,2,4a,5,6,7,8,8a-octahydronapthalene-1-carbaldehyde (79).[132]

Into a 25 mL round bottom flask with a magnetic stirrer bar was placed IBX (7.21×10^{-4} mol, 130 mg) and DMSO (5 mL). This mixture was stirred at room temperature until the IBX was fully dissolved (~30 minutes). Then, the decalin alcohol **55** (3.56×10^{-4} mol, 64.2 mg) was added. This mixture was stirred for 2

hours at room temperature. To quench the reaction, the reaction flask was cooled to 0 °C and water (10 mL) was added. The solution was stirred for 25 minutes at 0 °C, then the white precipitate was removed by vacuum filtration. The solution was transferred to a 60 mL separatory funnel and the aqueous solution was extracted with Et_2O (5x10 mL). The combined organic extracts were washed sequentially with water (2x10 mL), brine (2x10 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo*. The product was purified by flash chromatography (SiO_2, isocratic elution, 3% Et_2O:pentane). This provided decalin aldehyde **79** ($1.9x10^{-4}$ mol, 33.9 mg) as a volatile oil in a yield of 53%.

R_f 0.50 (10% EtOAc:hexanes); IR (CH_2Cl_2 cast) 3013, 2962, 2923, 2853, 2711, 1723, 1652 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.76 (d, 1H, J = 4.3 Hz, H9), 5.55 (ddd,1H, J = 2.5, 4.0, 10.0 Hz, H3), 5.43 (d, 1H, J = 10.0 Hz, H4), 2.63-2.56 (m, 1H, H2), 2.39 (dddd, 1H, J = 4.3, 5.0, 5.5, 5.7 Hz, H1), 1.85-1.60 (m, 6H, H4a,H5,H7,H8,H8a), 1.40-1.31 (m, 2H, H6), 1.61-1.09 (m, 1H, H7), 1.06 (d, 3H, J = 7.2 Hz, C$\underline{H}_3$2), 1.04-0.98 (m, 1H, H8); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 206.9 (C9), 131.2 (C4), 130.9 (C3), 55.5 (C1), 42.1 (C4a), 35.6 (C8a), 33.0 (C7), 31.7 (C2), 30.2 (C8), 26.6 (C5), 26.4 (C6), 17.0 (C$\underline{H}_3$2); HRMS (EI) calcd for $C_{12}H_{18}O$ 178.1358, found 178.1353 (M^+)

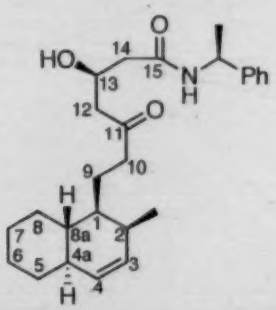


(±)(*R*,*E*)-13-(*tert*-Butyldimethylsilyloxy)-9-((1*S*,2*S*,4a*R*,8a*S*)-2-methyl-1,2,4a,5,6,7,8,8a-octahydronapthalen-1-yl)-11-oxo-*N*-((*S*)-1-phenylethyl)hept-10-enamide (80).

A 5 dram vial with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this vial was placed amide phosphonate **70** (1.99×10^{-4} mol, 100.1 mg), DBU (1.99×10^{-4} mol, 29.8 μL), LiCl (1.18×10^{-4}, ~5 mg) and CH_3CN (2 mL). The reaction mixture was stirred at room temperature for 30 minutes. At this time, the decalin aldehyde **79** (1.66×10^{-4} mol, 29.6 mg) was added via syringe. The reaction mixture was stirred at room temperature, under Ar for two days. Then, CH_3CN was removed *in vacuo*. The product was purified by flash chromatography (SiO_2, gradient elution, 10% EtOAc:hexanes, 30% EtOAc:hexanes, 100% EtOAc) to provide α,β-unsaturated ketone **80** (4.26×10^{-5} mol, 22.3 mg) as a viscous oil in a 27% yield.

R_f 0.30 (30% EtOAc:hexanes); IR ($CHCl_3$, cast) 3299, 3063, 3010, 2956, 2927, 2855, 1644, 1542 cm-1; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.35-7.22 (m, 5H, ArH), 6.81-6.68 (m, 2H, H9,NH), 6.02-5.97 (m, 1H, H10), 5.58-5.50 (m, 1H, H3), 5.45-5.39 (m, 1H, H4), 5.20-5.10 (m, 1H, NHCH(CH_3)), 4.60-4.53 (m, 1H, H13), 2.68-

2.64 (m, 2H, H12), 2.60-2.51 (m, 1H, H14), 2.38-2.24 (m, 2H, H14), 2.16-2.07 (m, 2H, H1,H2), 1.80-1.60 (m, 5H), 1.49-1.46 (m, 3H, NHCH($C\underline{H}_3$)), 1.44-1.36 (m, 1H, H8a), 1.34-1.20 (m, 3H), 1.10-1.07 (m, 3H, $C\underline{H}_3$2), 0.85 (s, 9H, SiC($C\underline{H}_3$)$_3$), 0.12-0.11 (m, 3H, Si$C\underline{H}_3$), 0.06-0.05 (m, 3H, Si$C\underline{H}_3$); HRMS (+ES) calcd for $C_{32}H_{49}NO_3SiNa$ 546.3374, found 546.3374 (M^++Na)

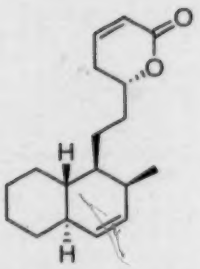


(±)(*R*)-13-Hydroxy-9-((1*S*,2*S*,4a*R*,8a*S*)-2-methyl-1,2,4a,5,6,7,8,8a-octahydronapthalen-1-yl)-11-oxo-*N*-((*S*)-1-phenylethyl)heptanamide (81).

A 5 mL round bottom flask with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this flask was placed α,β-unsaturated ketone **80** ($3.34x10^{-5}$ mol, 17.5 mg), Wilkinson's catalyst ($6.68x10^{-7}$ mol, or 0.37 mL of a 1.8 mM solution in benzene), Et_3SiH ($1.0x10^{-3}$ mol, 0.16 mL) and benzene (0.4 mL). The solution was heated to 65 °C and stirred for 7 hours. The reaction mixture was allowed to cool to room temperature then water (13.5 mL) and sat. $NaHCO_3$ (3.5 mL) was added to the reaction flask. The solution was transferred to a 60 mL separatory funnel and extracted with EtOAc (3x10 mL). The combined organic extracts were washed sequentially with water (10 mL), brine (10 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo*. The

crude residue, in a 1 dram vial, was dissolved in THF (0.1 mL) and stirred with a magnetic stirrer bar until it was completely dissolved. Then, glacial AcOH (0.1 mL) and TBAF (10 μL of 1 M solution in THF) were added to the reaction vial and stirred at room temperature for 3 days. The solution was diluted with water (10 mL) and transferred to a 60 mL separatory funnel by rinsing with EtOAc (10 mL). The organic layer was removed and the aqueous phase was extracted with EtOAc (2x10 mL). The combined organic extracts were washed sequentially with sat. $NaHCO_3$ (2x5 mL), brine (10 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo*. The residue was adsorbed onto SiO_2 and purified by flash chromatography (SiO_2, gradient elution, 30% EtOAc:hexanes, 50% EtOAc:hexanes, 100% EtOAc) . This provided compound **81** ($2.92x10^{-5}$ mol, 12 mg) as a viscous oil in a yield of 87%.

R_f 0.42 (60 % EtOAc:hexanes); IR (CH_2Cl_2 cast) 3307, 3063, 3006, 2923, 2854, 1710, 1643, 1544 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.36-7.31 (m, 4H, *o,m* ArH), 7.28-7.25 (m, 1H, *p* ArH), 6.43-6.41 (m, 1H, NH), 5.45-5.42 (m, 1H, H3), 5.39-5.37 (m, 1H, H4), 5.13 (m, 1H, NHCH(CH_3)), 4.41-4.37 (m, 1H, H13), 2.64-2.33 (m, 6H, H10,H12,H14), 2.04-1.99 (m, 1H, H2), 1.86-1.67 (m, 6H), 1.50-1.49 (m, 3H, NHCH(CH_3)), 1.37-1.25 (m, 4H), 1.12-1.10 (m, 1H), 1.03-1.02 (m, 3H, $CH_3$2), 1.01-0.80 (m, 3H); HRMS (+ES) calcd for $C_{26}H_{37}NO_3Na$ 434.2666, found 434.2666 (M^++Na)

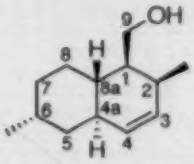


(±)-(*R*)-6-(2-((1*S*,2*S*,4a*R*,8a*S*)-2-Methyl-1,2,4a,5,6,7,8,8a-octahydronapthalen-1-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (82).

A 10 mL round bottom flask with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this flask was placed THF (0.4 mL) and CH_3OH (0.1 mL). This solution was cooled to -78 °C (dry ice/acetone) and then Et_3B ($4.03x10^{-5}$ mol, 41 μL) was added and the solution was stirred for 1.5 hours at -78 °C. Then compound **81** ($3.5x10^{-5}$ mol, 14.4 mg) was dissolved in 4:1, THF:CH_3OH (0.1 mL) and added to the reaction. This was followed by $NaBH_4$ ($3.9x10^{-5}$ mol, 1 mg). The reaction mixture was stirred at -78 °C for 4 hours. The reaction was quenched by the addition of sat. NH_4Cl (0.5 mL). The solution was allowed to warm to room temperature at which time water (0.5 mL) was added until the solid dissolved. The mixture was transferred to a 30 mL separatory funnel and extracted with EtOAc (3x5 mL). The combined organic extracts were dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was dissolved in CH_3OH (10 mL). The solution was heated at 50 °C for one hour. The solution was allowed to cool to room temperature then the solvent was removed *in vacuo*. The residue was dissolved in benzene (3 mL), *p*-TsOH ($2.63x10^{-5}$ mol, 5 mg) was added and the mixture was heated at 50 °C overnight.

The solution was allowed to cool to room temperature and then was neutralized with solid $NaHCO_3$. The mixture was transferred to a 30 mL separatory funnel using EtOAc (5x2 mL). The organic phase was washed sequentially with water (2x5 mL), brine (5 mL) and dried over Na_2SO_4. The product was purified by flash chromatography (SiO_2, gradient elution, 100% hexanes, 20% EtOAc:hexanes). This provided the desmethyl diene **82** ($2.55x10^{-5}$ mol, 7 mg) as a viscous oil in a yield of 73%.

R_f 0.63 (50% EtOAc:hexanes); IR ($CHCl_3$ cast) 2923, 2853, 1735, 1464 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 6.90-6.86 (m, 2H), 6.04-6.02 (m, 2H), 5.46-5.39 (m, 2H), 5.39-5.37 (m, 2H), 4.44-4.37 (m, 2H), 2.39-2.28 (m, 4H), 2.11-2.07 (m, 2H), 1.97-1.91 (m, 2H), 1.80-1.65 (m, 5H), 1.59-1.49 (m, 8H), 1.41-1.23 (m, 6H), 1.19-1.10 (m, 5H),1.05 (d, 3H, J = 7.2 Hz),1.04 (d, 3H, J = 7.2 Hz),1.01-0.82 (m, 4H); HRMS (EI) calcd for $C_{18}H_{26}O_2$ 274.1933, found 274.1922 (M^+)

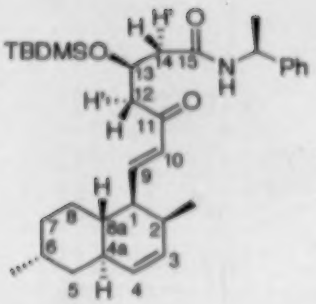


((1*S*,2*S*,4a*R*,6*R*,8a*S*)-2,6-Dimethyl)-1,2,4a,5,6,7,8,8a-octahydronapthalen-1-yl)methanol (84).[40, 135]

A 25 mL round bottom flask with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this flask was placed 6-Me-decalin ethyl ester **83** supplied by Dr. Doug Burr ($5.5x10^{-4}$ mol, 129 mg) and THF (5 mL). This mixture was cooled to 0 °C and then $LiAlH_4$ ($5.5x10^{-4}$ mol, 0.55 mL of a 1M

solution in THF) was added dropwise to the stirred solution. The reaction was stirred for one hour during which time the solution was allowed to warm to room temperature. The solution was then cooled to 0 °C and quenched by the sequential addition of water (1 mL), NaOH (1 mL of 3 M solution) and water (2 mL). This solution was stirred vigorously at room temperature for one hour. Then, $MgSO_4$ (~0.5 g) was added to the stirred solution. The solid was removed by vacuum filtration and the solvent was removed *in vacuo*. The residue was purified using flash chromatography (SiO_2, isocratic elution, 10% EtOAc:hexanes). The 6-Me-decalin alcohol **84** was obtained (5.08×10^{-4} mol, 98.6 mg) as a white solid in a 92% yield.

R_f 0.12 (10% EtOAc:hexanes); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.61 (ddd, 1H, J = 2.5, 9.7, 14.5 Hz, H3), 5.31 (d, 1H, J = 9.7 Hz, H4), 3.85 (dd, 1H, J = 5.0, 10.5 Hz, H9), 3.55 (dd, 1H, J = 9.5, 10.5 Hz, H9), 2.46-2.40 (m, 1H, H2), 2.08-2.00 (m, 1H, H6), 2.00-1.92 (m, 1H, H4a), 1.78 (dddd, 1H, J = 5.0, 5.5, 9.0, 10.3 Hz, H1), 1.56-1.47 (m, 4H, H7,H8,H5), 1.48-1.19 (m, 3H, O<u>H</u>, H5,H8), 1.10-1.04 (m, 1H, H8a), 1.01 (d, 3H, J = 7.0 Hz, C<u>H</u>$_3$6), 0.93 (d, 3H, J = 7.5 Hz, C<u>H</u>$_3$2); ($CDCl_3$, 125 MHz) δ 132.5 (C3), 131.5 (C4), 63.1 (C9), 44.1 (C1), 38.8 (C5), 38.3 (C8a), 36.9 (C4a), 32.1 (C7), 31.6 (C2), 27.5 (C6), 23.8 (C8), 18.1 (C6<u>C</u>H$_3$), 15.6 (C2<u>C</u>H$_3$)

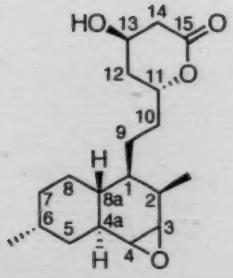


(*R*,*E*)-13-(*tert*-Butyldimethylsilyloxy)-9-((1*S*,2*S*,4a*R*,6*R*,8a*S*)-2,6-dimethyl-1,2,4a,5,6,7,8,8a-octahydronapthalen-1-yl)-11-oxo-*N*-((S)-15-phenylethyl)-hept-10-enamide (85).[84, 85, 88]

A 1 dram vial with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this vial was placed amide phosphonate **70** ($7x10^{-4}$ mol, 33 mg, in 0.38 mL CH_3CN), LiCl ($7x10^{-4}$ mol, ~3 mg) and DBU ($7x10^{-4}$ mol, 10 μL). This mixture was stirred at room temperature for 30 minutes. Then a solution of 6-Me-decalin aldehyde **79** ($5.84x10^{-4}$ mol, 11.2 mg, in 0.2 mL of CH_3CN) was added to the vial. The volume of the reaction mixture was increased to 1 mL with additional CH_3CN. The reaction mixture was stirred for 16 days at room temperature under Ar. The CH_3CN was removed *in vacuo* and the residue was transferred from the vial to a 25 mL separatory funnel using EtOAc (3x3 mL) and water (3x1 mL). The aqueous layer was extracted with additional EtOAc (3x3 mL) and the organic layers were combined. The combined organic extracts were washed sequentially with saturated NH_4Cl (5 mL), water (5 mL), brine (10 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the product was purified using flash chromatography (SiO_2, gradient elution 5%

EtOAc:hexanes, 10% EtOAc:hexanes, 100% EtOAc) to give 6Me-α,β-unsaturated ketone **85** ($1.3x10^{-4}$ mol, 7 mg) as a viscous oil in a yield of 22%.

R_f 0.40 (40% EtOAc: hexanes); IR ($CHCl_3$ cast) 3301, 3063, 2928, 1699, 1646, 1541 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.36-7.32 (m, 4H, *o,m* ArH), 7.27-7.25 (m, 1H, *p* ArH), 6.80 (d, 1H, *J* = 7.8 Hz, NH), 6.65 (dd, 1H, *J* = 10.5, 15.6 Hz, H9), 6.01 (dd, 1H, *J* = 0.6, 15.6 Hz, H10), 5.58 (ddd, 1H, *J* = 3.0, 4.8, 9.6 Hz, H3), 5.35 (d, 1H, *J* = 9.6 Hz, H4), 5.16 (dq, 1H, *J* = 6.4, 7.8, Hz, NHCH(CH_3)), 4.59 (dddd, 1H, *J* = 4.8, 4.8, 5.4, 5.4 Hz, H13), 2.67 (dd, 1H, *J* = 5.4, 15.6 Hz, H12), 2.64 (dd, 1H, *J* = 4.8, 15.6 Hz, H12'), 2.56 (dd, 1H, *J* = 5.4, 15.0 Hz, H14), 2.34 (dd, 1H, *J* = 4.8, 15.0 Hz, H14'), 2.32-2.29 (m, 1H, H1), 2.28-2.22 (m, 1H, H2), 2.10-2.04 (m, 1H, H6), 1.96-1.90 (m, 1H, H4a), 1.58-1.50 (m, 3H, H5,H7), 1.47 (d, 3H, *J* = 6.4 Hz, NHCH(CH_3)), 1.35-1.17 (m, 3H, H5,H8,H8a), 1.09-1.02 (m, 1H, H8), 0.99 (d, 3H, *J* = 7.2 Hz, $CH_3$6), 0.90 (d, 3H, *J* = 6.0 Hz, $CH_3$2), 0.89 (s, 9H, SiC(CH_3)$_3$), 0.13 (s, 3H, Si(CH_3)), 0.07 (s, 3H, Si(CH_3)); ^{13}C NMR ($CDCl_3$ 125 MHz) δ 197.7 (C11), 168.7 (C15), 150.8 (C9), 143.7 (C4°Ar), 132.0 (C3), 131.6 (C10), 131.4 (C4), 128.6 (*ortho*), 127.2 (*meta*), 126.2 (*para*), 66.6 (C13), 48.5 (NHCH(CH_3)), 47.5 (C1), 45.8 (C12), 43.9 (C14), 39.1 (C8a), 38.7 (C5), 36.2 (C4a), 35.7 (C2), 32.0 (C7), 27.6 (C6), 25.8 (SiC(CH_3)$_3$), 25.4 (C8), 22.1 (NHCH(CH_3)), 18.2 (C6CH_3), 17.9 (SiC(CH_3)$_3$), 16.6 (C2CH_3), -4.9 (Si(CH_3)$_2$); HRMS (+ES) calcd for $C_{33}H_{51}NO_3SiNa$ 560.3530, found 560.3532 (M^++Na)

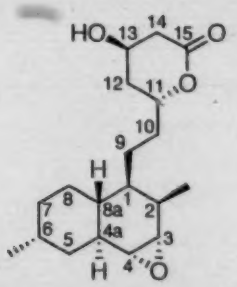


(4*R*,6*R*)-6-(2-((2*R*,3*R*,3a*R*,6*R*,7a*S*)-2,6-Dimethyldecahydronaptho[1,2-*b*]oxiren-3-yl)ethyl)-4-hydroxytetrahydro-2*H*-pyran-2-one (89 and90).[100]

The procedure used was the same as that used for the preparation of TBDPS epoxides **92** and **93**. From DHL **13** ($6.1x10^{-4}$ mol, 187 mg) the products DHL epoxides **89** and **90** ($5.52x10^{-4}$ mol, 178 mg) were isolated as a white solid in a yield of 91%. Analysis of the ^{1}H NMR spectrum revealed that the ratio of α:β was 1:2. The pure α-DHL epoxide **90** (37 mg) was isolated by flash chromatography (SiO_2, isocratic elution, 30% EtOAc:hexanes) as a white solid. The data provided is for the mixture of stereoisomers.

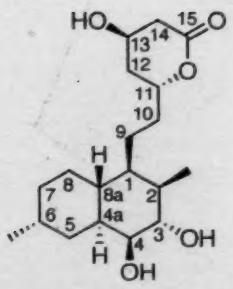
R_f 0.16 (70% EtOAc:hexanes); IR (microscope) 3388, 2999, 2956, 2937, 2914, 2876, 1666 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.70-4.64 (m, 2H), 4.47-4.34 (m, 2H), 3.19 (dd, 1H, *J* = 4.2, 4.2 Hz, H3β), 3.00 (d, 1H, *J* = 3.6 Hz, H3α), 2.94 (d, 1H, *J* = 4.2 Hz, H4β), 2.78-2.72 (m, 2H, H14), 2.75 (d, 1H, *J* = 3.6 Hz, H4α), 2.65-2.59 (m, 2H, H14'), 2.37-2.30 (m, 1H, H2α), 2.20-2.15 (m, 1H, H2β), 2.10-2.06 (m, 2H), 1.98-1.92 (m, 2H), 1.82-1.68 (m, 6H), 1.67-1.36 (m, 16H), 1.34-1.20 (m, 3H), 1.05-0.90 (m, 2H), 1.00 (d, 3H, *J* = 7.1 Hz, $C\underline{H}_3$α), 0.99 (d, 3H, *J* =

6.8 Hz, C<u>H</u>$_3$β), 0.95 (d, 3H, *J* = 7.3 Hz, C<u>H</u>$_3$β), 0.92 (d, 3H, *J* = 7.3 Hz, C<u>H</u>$_3$α), 0.89-0.80 (m, 1H); HRMS (EI) calcd for $C_{19}H_{30}O_4$ 322.2144, found 322.2140 (M^+)



(4*R*,6*R*)-6-(2-((1a*S*,2*R*,3*R*,3a*R*,6*R*,7a*S*,7b*R*)-2,6-Dimethyldecahydronaptho-[1,2-*b*]oxiren-3-yl)ethyl)-4-hydroxytetrahydro-2*H*-pyran-2-one (90).[100]

R_f 0.16 (70% EtOAc:hexanes); $[\alpha]_D^{25}$ 90° (*c* 0.95, $CHCl_3$); IR ($CHCl_3$ cast) 3392, 2915, 2876, 1709 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.68-4.63 (m, 1H), 4.41-4.38 (m, 1H), 3.00 (dd, 1H, *J* = 1.2, 3.7 Hz, H3), 2.7483 (d, 1H, *J* = 3.8 Hz, H4), 2.7478 (dd, 1H, *J* = 5.0, 17.6 Hz, H14), 2.62 (ddd, 1H, *J* = 1.6, 3.7, 17.6 Hz, H14'), 2.37-2.30 (m, 1H, H2), 2.12-2.04 (m, 1H, H6), 1.98-1.92 (m, 1H), 1.80-1.38 (m, 11H), 1.38-1.25 (m, 1H), 1.02-0.96 (m, 1H), 0.99 (d, 3H, *J* = 7.2 Hz), 0.92 (d, 3H, *J* = 7.3 Hz), 0.88-0.81 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.4, 76.0, 62.8, 58.8, 57.5, 38.6, 38.8, 37.4, 36.3, 36.2, 36.1, 33.0, 32.0, 30.3, 27.6, 23.9, 23.7, 18.1, 10.2; HRMS (EI) calcd for $C_{19}H_{30}O_4$ 322.2144, found 322.2145 (M^+)

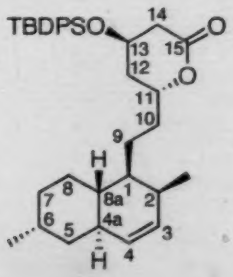


(4*R*,6*R*)-6-((1*R*,2*R*,3*S*,4*S*,4a*S*,6*R*,8a*R*)-3,4-Dihydroxy-2,6-dimethyldecahydronapthalen-1-yl)ethyl)-4-hydroxytetrahydro-2*H*-pyran-2-one (91).

Into an NMR tube was placed a solution of DHL epoxides **89** and **90** (4.03×10^{-6} mol, 1.3 mg in 0.5 mL of CD_2Cl_2) and TFA (4.03×10^{-6} mol, 20 μL). The tube was fitted with a rubber septum and flushed out with Ar (~3 minutes). The reaction was allowed to proceed at room temperature and followed by ^1H NMR. After 3 days, all of the starting material was consumed. The mixture was transferred to a 30 mL separatory funnel using CH_2Cl_2 (10 mL). The organic phase was washed sequentially with water (2x2 mL), brine (2 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo*. The product was purified by flash chromatography (SiO_2, isocratic elution, 70% EtOAc:hexanes). The triol **91** (2.35×10^{-6} mol, 0.8 mg) was obtained as a white solid with a yield of 58%.

R_f 0.24 (80% EtOAc:hexanes); ^1H NMR ($CDCl_3$, 500 MHz) δ 4.91-4.89 (m, 1H), 4.73-4.67 (m, 1H), 3.89-3.87 (m, 1H), 2.76 (dd, 1H, J = 5.0, 17.5 Hz), 2.63 (ddd, 1H, J = 1.5, 3.6, 17.5 Hz), 2.10-1.93 (m, 4H), 1.82-1.66 (m, 7H), 1.58-1.41 (m,

2H), 1.38-1.31 (m, 2H), 1.27-1.21 (m, 1H), 1.14-1.05 (m, 1H), 1.00 (d, 3H, J = 7.2 Hz), 0.94 (d, 3H, J = 7.6 Hz)



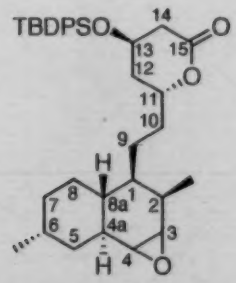
(4*R*,6*R*)-4-(*tert*-Butyldiphenylsilyloxy)-6-(2-((1*S*,2*S*,4a*R*,6*R*,8a*S*)-2,6-dimethyl-1,2,4a,5,6,7,8,8a-octahydronapthalen-1-yl)ethyl)tetrahydro-2*H*-pyran-2-one (92a).

A 10 mL round bottom flask with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this flask was placed DHL **13** (3.92×10^{-4} mol, 120.1 mg), imidazole (7.84×10^{-4} mol, 54 mg), TBDPS-Cl (3.92×10^{-4} mol, 0.1 mL) and DMF (2 mL). This solution was then placed in an oil bath at 40 °C and stirred overnight. Then the solvent was removed *in vacuo*. The product was purified by passing through a small plug of silica gel using 40% EtOAc:hexanes to elute the protected DHL. This provided TBDPS-DHL **92a** (3.53×10^{-4} mol, 192 mg) as a viscous oil in a yield of 90%.

R_f 0.44 (40% EtOAc:hexanes); $[\alpha]_D^{25}$ 22.2° (*c* 0.11, $CHCl_3$); IR ($CHCl_3$ cast) 3071, 3050, 3011, 2958, 2930, 2858, 1734, 1653, 1590, 1559, 1472 cm^{-1};

^1H NMR ($CDCl_3$, 300 MHz) δ 7.74-7.70 (m, 2H), 7.65-7.61 (m, 4H), 7.48-7.36 (m, 4H), 5.60 (ddd, 1H, J = 2.7, 4.8, 9.8 Hz), 5.31 (d, 1H, J = 9.8 Hz), 4.82-4.73 (m,

1H), 4.32-4.27 (m, 1H), 2.62 (ddd, 1H, J = 1.7, 3.3, 17.5 Hz), 2.47 (dd, 1H, J = 4.5, 17.5 Hz), 2.43-2.17 (m, 1H), 2.07-2.00 (m, 1H), 1.96-1.87 (m, 1H), 1.82-1.69 (m, 1H), 1.62-1.43 (m, 7H), 1.40-1.20 (m, 4H), 1.08 (s, 9H), 1.00 (d, 3H, J = 7.2 Hz), 0.84 (d, 3H, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.3, 135.53, 135.52, 134.7, 132.5, 131.5, 130.0, 129.9, 127.79, 127.76, 127.6, 76.3, 64.4, 41.4, 39.9, 38.92, 38.86, 37.2, 36.0, 33.2, 32.2, 31.9, 27.4, 26.8, 26.5, 23.59, 23.57, 19.0, 18.1, 14.9; HRMS (EI) calcd for $C_{31}H_{39}O_3Si$ 487.2669, found 487.2668 (M^+-*tert*-butyl)

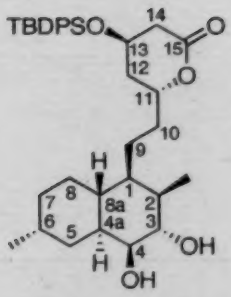


(4*R*,6*R*)-(*tert*-Butyldiphenylsilyloxy)-6-(2-((2*R*,3*R*,3a*R*,6*R*,7a*S*)-2,6-dimethyldecahydronaptho[1,2-*b*]oxiren-3-yl)ethyl)tetrahydro-2*H*-pyran-2-one (92 and 93).

A 50 mL round bottom flask with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this flask was placed TBDPS-DHL **92a** ($2.52x10^{-4}$ mol, 137 mg), CH_2Cl_2 (20 mL) and *m*-CPBA ($2x10^{-3}$ mol, 346 mg). This solution was stirred at room temperature for 4 hours. The reaction mixture was then transferred to a 60 mL separatory funnel. The organic layer was washed sequentially with $NaHSO_3$ (15 mL of a 6% solution), saturated $NaHCO_3$

(15 mL), brine (15 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo*. The product was purified by using flash chromatography (SiO_2, isocratic elution, 40% EtOAc:hexanes). This provided the TBDPS epoxides **92** and **93** ($2.50x10^{-4}$ mol, 140 mg) as a white solid in a quantitative yield. Analysis of the products **92** and **93** by ^1H NMR indicated that the ratio of α:β epoxide was 3:2. The data provided is for the mixture of stereoisomers.

R_f 0.74 (40% EtOAc:hexanes); IR (CH_2Cl_2 cast) 3071, 2959, 2931, 2857, 1739, 1472, 1462 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.64-7.61 (m, 4H, *o* ArH), 7.47-7.44 (m, 2H, *p* ArH), 7.41-7.38 (m, 4H, *m* ArH), 4.78-4.70 (m, 2H, H11), 4.31-4.27 (m, 2H, H13), 3.18 (dd, 1H, *J* = 3.9, 5.6 Hz, H3β), 2.99 (dd, 1H, *J* = 1.4, 3.8 Hz, H3α), 2.94 (d, 1H, *J* = 3.9 Hz, H4β), 2.74 (d, 1H, *J* = 3.8 Hz, H4α), 2.61 (ddd, 2H, *J* = 3.0, 5.0, 17.4 Hz, H14'α,14'β), 2.463 (dd, 1H, *J* = 4.6, 17.4 Hz, H14β), 2.460 (dd, 1H, *J* = 4.6, 17.4 Hz, H14α), 2.34-2.27 (m, 1H, H2α), 2.18-2.11 (m, 1H, H2β), 2.11-2.04 (m, 2H, H6), 1.80-1.42 (m, 19H), 1.41-1.3 (m, 4H), 1.26-1.18 (m, 2H), 1.16-0.90 (m, 14H), 1.07 (s, 18H, $SiC(C\underline{H}_3)_3$), 1.00 (d, 3H, *J* = 7.1 Hz, $C\underline{H}_3$6α), 0.99 (d, 3H, *J* = 7.2 Hz, $C\underline{H}_3$6β), 0.93 (d, 3H, *J* = 7.1 Hz, $C\underline{H}_3$2β), 0.91 (d, 3H, *J* = 7.2 Hz, $C\underline{H}_3$2α), 0.88-0.80 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.3 (C15α), 170.3 (C15β), 135.6, 133.3, 133.03, 133.00, 130.09, 130.07, 130.06, 127.89, 127.87, 77.2, 76.5, 76.1, 64.5, 60.4, 58.7, 58.2, 57.5, 57.0, 41.6, 39.0, 38.4, 37.7, 37.4, 36.4, 36.22, 36.19, 35.9, 35.6, 34.6, 33.3, 33.1, 32.1, 31.7, 30.3, 29.0, 27.6, 27.0, 26.9, 23.9, 23.8, 23.3, 22.7, 21.0, 19.1, 18.2, 18.0, 14.2, 10.3, 9.6; HRMS (+ES) calcd for $C_{35}H_{48}O_4SiNa$ 583.3214, found 583.3217 (M^++Na)

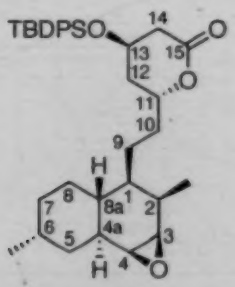


(4*R*,6*R*)-4-(*tert*-Butyldiphenylsilyloxy)-6-(2-((1*R*,2*R*,3*S*,4*S*,4a*S*,6*R*,8a*R*)-3,4-dihydroxy-2,6-dimethyldecahydronapthalen-1-yl)ethyl)tetrahydro-2*H*-pyran-2-one (94).

Into a 25 mL round bottom flask with a magnetic stirrer bar was placed TBDPS epoxides **92** and **93** (2.68×10^{-4} mol, 151 mg), CH_3CN (10 mL), milliQ purified water (1 mL) and TFA (15 μL). The flask was fitted with a rubber septum and stirred at room temperature under Ar overnight. The solvent was removed *in vacuo*. The product was purified using flash chromatography (SiO_2, gradient elution, 10% EtOAc:hexanes, 100% EtOAc). This provided the TBDPS diol **94** (1.31×10^{-4} mol, 75.6 mg) as a white solid in a yield of 48%.

R_f 0.43 (70% EtOAc:hexanes); $[\alpha]_D^{25}$ 1.04° (*c* 0.44, $CHCl_3$); IR (cast) 3446, 2954, 2917, 2849, 1735, 1473 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.65-7.62 (m, 4H, *o* ArH), 7.48-7.45 (m, 2H, *p* ArH), 7.41-7.38 (m, 4H, *m* ArH), 4.79-4.74 (m, 1H, H11), 4.31-4.28 (m, 1H, H13), 3.86 (dd, 1H, *J* = 2.6, 2.6 Hz, H3), 3.56 (dd, 1H, *J* = 2.0, 2.0 Hz, H4), 2.61 (ddd, 1H, *J* = 1.8, 3.2, 17.5 Hz, H14'), 2.47 (dd, 1H, *J* = 4.6, 17.5 Hz, H14), 2.14-2.07 (m, 1H, H6), 1.94-1.88 (m, 1H, H2), 1.80-1.74 (m, 2H, H4a,H12'), 1.73-1.30 (m, 14H), 1.29-1.22 (m, 2H), 1.08-1.07 (m, 1H),

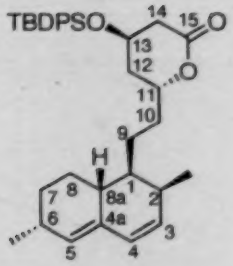
1.08 (s, 9H, SiC($C\underline{H}_3$)$_3$), 1.013 (d, 3H, J = 7.7 Hz, $C\underline{H}_3$2), 1.009 (d, 3H, J = 7.1 Hz, $C\underline{H}_3$6); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.4 (C15), 135.63 (C*ortho*), 135.62 (C*ortho*), 133.3 (C4°), 133.0 (C4°), 130.1 (C*meta*), 130.0 (C*meta*), 127.89 (C*para*), 127.87 (C*para*), 76.5 (C11), 75.4 (C3), 75.2 (C4), 64.5 (C13), 39.3, 39.0 (C14), 36.7 (C2), 36.0 (C12), 35.3, 34.7, 34.2, 33.0, 31.6, 27.2, 26.9 (SiC($\underline{C}H_3$)$_3$), 24.2, 23.9, 19.1, 17.8 ($\underline{C}H_3$6), 11.9 ($\underline{C}H_3$2); HRMS (+ES) calcd for $C_{35}H_{50}O_5SiNa$ 601.3320, found 601.3320 (M^++Na)



(4*R*,6*R*)-4-(*tert*-Butyldiphenylsilyloxy)-6-(2-((1a*R*,2*R*,3*R*,3a*R*,6*R*,7a*S*,7b*S*)-2,6-dimethyldecahydronaptho[1,2-*b*]oxiren-3-yl)tetrahydro-2*H*-pyran-2-one (92 and 93).

A 10 mL round bottom flask with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this flask was placed triphenylphosphine oxide (1.36×10^{-4} mol, 36 mg), THF (2 mL) and TBDPS diol **94** (1.24×10^{-4} mol, 72.2 mg). The solution was stirred until the reagents dissolved. Then, DEAD (1.40×10^{-4} mol, 22 μL) was added in one portion and the solution was stirred at room temperature overnight. The mixture was adsorbed onto silica gel and subjected to flash chromatography (SiO_2, isocratic elution, 20% EtOAc:hexanes).

This provided the TBDPS epoxides **92** and **93** (6.83×10^{-5} mol, 38.3 mg) as a viscous oil in a yield of 55%. Analysis of the epoxides by ^{1}H NMR revealed that it was a 1:4 mixture of α:β. The spectral data matched those reported for TBDPS epoxides **92** and **93** in the previous procedure. However, since the ratio is in favor of the β-TBDPS epoxide **92** the data presented is for the β stereoisomer **92**. R_f 0.40 (30% EtOAc:hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.64-7.61 (m, 8H), 7.48-7.44 (m, 4H), 7.42-7.38 (m, 8H), 4.78-4.72 (m, 2H), 4.31-4.28 (m, 2H), 3.18 (dd, 1H, *J* = 3.5, 5.6 Hz), 3.00 (m, 1H), 2.94 (d, 1H, *J* = 3.5 Hz), 2.75 (m, 1H), 2.64-2.58 (m, 2H), 2.50-2.44 (m, 2H), 2.33-2.23 (m, 1H), 2.18-2.05 (m, 4H), 1.80-1.58 (m, 8H), 1.56-1.45 (m , 13H), 1.44-1.32 (m, 4H), 1.28-1.11 (m, 4H), 1.08 (s, 18H), 1.06-0.98 (m, 2H), 1.01 (d, 3H, *J* = 7.0 Hz), 1.00 (d, 3H, *J* = 7.2 Hz), 0.94 (d, 3H, *J* = 7.1 Hz), 0.91 (d, 3H, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.3, 135.63, 135.61, 133.3, 133.0, 130.08, 130.05, 127.90, 127.87, 76.1, 64.5, 58.2, 57.0, 41.6, 39.0, 37.7, 35.9, 35.6, 34.6, 33.3, 31.7, 29.0, 27.0, 26.9, 23.3, 22.7, 19.1, 18.0, 9.6

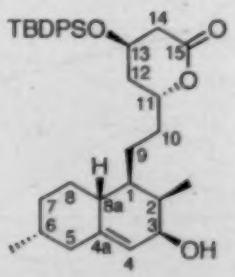


(4*R*,6*R*)-4-(*tert*-Butyldiphenylsilyloxy)-6-(2-((1*S*,2*S*,6*R*,8a*R*)-2,6-dimethyl-1,2,6,7,8,8a-hexahydronapthalen-1-yl)ethyl)tetrahydro-2*H*-pyran-2-one (95).

A 10 mL round bottom flask with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this flask was added triphenylphosphine oxide (3.89×10^{-4} mol, 108 mg) and 1,2-DCE (0.5 mL). This solution was cooled to 0 °C. Then, triflic anhydride (1.95×10^{-4} mol, 33 μL in 0.2 mL of 1,2-DCE) was added via syringe. A white solid formed immediately and the reaction was stirred at 0 °C for 10 minutes. A solution of TBDPS epoxides **92** and **93** (1.95×10^{-4} mol, 109 mg in 0.3 mL of 1,2-DCE) were added followed by NEt_3 (1.95×10^{-4} mol, 27 μL). The solution was stirred at room temperature for 3 days. The solvent was removed *in vacuo*. The product was purified by flash chromatography ($AgNO_3$•SiO_2, isocratic elution, 5% EtOAc:15% CH_2Cl_2:85% hexane) to provide a viscous oil (13.3 mg) in which the TBDPS-monacolin L **95** was the major product in a yield of 12%.

R_f 0.74 (30% EtOAc:hexanes); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.65-7.61 (m, 8H), 7.48-7.38 (m, 12H), 5.92 (d, 1H, J = 9.8 Hz), 5.72 (dd, 1H, J = 6.0, 9.8 Hz), 5.45-5.43 (m, 1H), 4.80-4.72 (m, 2H), 4.32-4.26 (m, 2H), 2.64-2.58 (m, 2H), 2.50-2.45

(m, 2H), 2.37-2.25 (m, 2H), 2.23-2.17 (m, 1H), 2.09-1.98 (m, 2H), 1.90-1.64 (m, 13H), 1.62-1.46 (m, 11H), 1.44-1.30 (m, 4H), 1.22-1.14 (m, 2H), 1.08 (m, 18H), 1.02 (d, 1H, J = 6.8 Hz), 1.00 (d, 3H, J = 7.1 Hz), 0.89 (d, 3H, J = 7.0 Hz), 0.73 (d, 1H, J = 6.5 Hz)

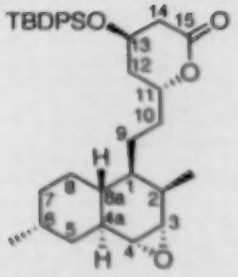


(4*R*,6*R*)-4-(*tert*-Butyldiphenylsilyloxy)-6-(2-((1*R*,2*R*,3*R*,6*R*,8a*R*)-3-hydroxy-2,6-dimethyl-1,2,3,5,6,7,8,8a-octahydronapthalen-1-yl)ethyl)tetrahydro-2*H*-pyran-2-one (96).[113, 115]

A 100 mL round bottom flask with a magnetic stirrer bar was fitted with a reflux condenser and flame dried under Ar. Into this flask was placed TBDPS epoxides **92** and **93** (1.77×10^{-4} mol, 99.1 mg), $Al(O/Pr)_3$ (1.77×10^{-4} mol, 36 mg) and toluene (40 mL). The solution was heated at reflux overnight. Then the solution was allowed to cool to room temperature and transferred to a 125 mL separatory funnel. The mixture was washed with NaOH (20 mL of 2M solution) then brine until the pH of the washings was ~7. The solvent was removed *in vacuo*. The product was purified by flash chromatography (SiO_2, gradient elution, 10% EtOAc:hexanes, 30% EtOAc:hexanes). This provided the 3β-3,5-hydroxy-DHL **96** (1.78x10-6 mol, 1 mg) as a white solid in a yield of 0.01%. In addition, this

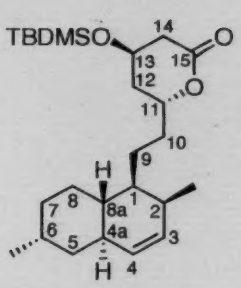
allowed the isolation of α-TBDPS epoxide **93** (2.14×10^{-6} mol, 1.2 mg) as a white solid in a yield of 0.01%.

R_f 0.34 (40% EtOAc:hexanes); $[\alpha]_D^{25}$ -7.50 (*c* 0.06, $CHCl_3$); IR ($CHCl_3$ cast) 3071, 2958, 2930, 1738, 1589, 1471, 1462 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.65-7.61 (m, 4H, *o* ArH), 7.47-7.44 (m, 2H, *p* ArH), 7.41-7.38 (m, 4H, *m* ArH), 5.45-5.44 (m, 1H, H4), 4.79-4.72 (m, 1H, H11), 4.31-4.27 (m, 1H, H13), 3.78-3.75 (m, 1H, H3), 2.60 (ddd, 1H, *J* = 1.9, 3.1, 17.6 Hz, H14'), 2.55-2.49 (m, 1H, H5'), 2.46 (dd, 1H, *J* = 4.6, 17.6 Hz, H14), 2.20-2.15 (m, 1H, H6), 2.07-2.03 (m, 1H, H5), 1.97-1.86 (m, 2H), 1.81-1.2 (m, 10H), 1.08 (s, 9H, $Si(C(CH_3)_3)$), 0.99 (d, 3H, *J* = 7.1 Hz, $CH_3$6), 0.87 (d, 3H, *J* = 7.3 Hz, $CH_3$2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.4 (C15), 135.6 (C*ortho*), 134.9 (C4a), 133.3 (C4°Ar), 133.0 (C4°Ar), 131.0 (C4), 130.08 (C*para*), 130.05 (C*para*), 127.89 (C*meta*), 127.87 (C*meta*), 76.4 (C11), 72..3 (C3), 64.5 (C13), 40.2, 39.0, 37.9, 36.5, 36.4, 36.2, 33.0, 29.7, 28.3, 26.9 ($SiC(CH_3)_3$), 24.9, 23.8, 21.3, 19.1 ($CH_3$6), 11.4 ($CH_3$2); HRMS (+ES) calcd for $C_{35}H_{48}O_4SiNa$ 583.3214, found 583.3217 (M^++Na)



(4*R*,6*R*)-(*tert*-Butyldiphenylsilyloxy)-6-(2-((1a*S*,2*R*,3*R*,3a*R*,6*R*,7a*S*,7b*R*)-2,6-dimethyldecahydronaptho[1,2-*b*]oxiren-3-yl)ethyl)tetrahydro-2*H*-pyran-2-one (93).

R_f 0.66 (40% EtOAc:hexanes); $[\alpha]_D^{25}$ 6.46 (*c* 0.12, $CHCl_3$); IR ($CHCl_3$ cast) 2956, 2917, 2849, 1738 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.65-7.62 (m, 4H), 7.48-7.45 (m, 2H), 7.41-7.38 (m, 4H), 4.76-4.70 (m, 1H), 4.30-4.28 (m ,1H), 3.00 (dd, 1H, *J* = 1.2, 3.8 Hz), 2.75 (d, 1H, *J* = 3.8 Hz), 2.61 (ddd, 1H, *J* = 1.9, 3.3, 17.5 Hz), 2.46 (dd, 1H, *J* = 4.5, 17.5 Hz), 2.34-2.28 (m, 1H), 2.12-2.05 (m, 1H), 1.80-1.75 (m, 1H), 1.69-1.44 (m, 7H), 1.40-1.20 (m, 6H), 1.26 (s, 9H), 1.01 (d, 3H, *J* = 7.2 Hz), 0.90 (d, 3H, *J* = 7.2 Hz), 0.90-0.80 (m, 1H); HRMS (+ES) calcd for $C_{35}H_{48}O_4SiNa$ 583.3214, found 583.3216 (M^++Na)

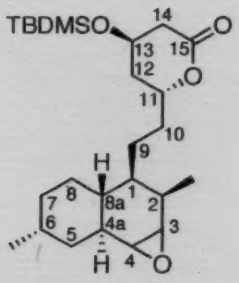


(4*R*,6*R*)-4-(*tert*-Butyldimethylsilyloxy)-6-(2-((1a*S*,2*R*,3*R*,3a*R*,6*R*,7a*S*,7b*R*)-2,6-dimethyldecahydronaptho[1,2-*b*]oxiren-3-yl)ethyl)tetrahydro-2*H*-pyran-2-one (100a).[118]

A 25 mL round bottom flask with a magnetic stirrer bar was fitted with a rubber septum and flame dried under Ar. Into this flask was placed DHL **13** (4.89×10^{-4} mol, 150 mg), CH_2Cl_2 (10 mL) and 2,6-lutidine (9.78×10^{-4} mol, 0.11 mL). This mixture was stirred and cooled down to -78 °C. Then, TBDMSOTf (7.34×10^{-4} mol, 0.17 mL) was added slowly via syringe. The reaction mixture was stirred for one hour at -78 °C. The reaction was quenched by the addition of water (1 mL) at -78 °C after which the solution was allowed to warm to room temperature. The solution was transferred to a 60 mL separatory funnel, washed with brine (10 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo*. The product was purified by flash chromatography (SiO_2, gradient elution, 10% EtOAc:hexanes, 80% EtOAc:hexanes). This provided TBDMS DHL **100a** (2.27×10^{-4} mol, 95.2 mg) as a white solid in a yield of 46%.

R_f 0.58 (30% EtOAc:hexanes); $[\alpha]_D^{25}$ -0.75° (*c* 0.63, $CHCl_3$); IR ($CHCl_3$ cast) 3007, 2955, 2928, 2857, 1741, 1692 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.60

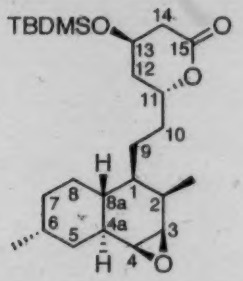
(dddd, 1H, J = 2.8, 4.8, 4.8, 5.0 Hz, H3), 5.31 (d, 1H, J = 9.9 Hz, H4), 4.70-4.65 (m, 1H, H11), 4.32-4.29 (m, 1H, H13), 2.63 (dd, 1H, J = 4.4, 17.3 Hz, H14), 2.59-2.55 (m, 1H, H14'), 2.26-2.20 (m, 1H, H2), 2.06-2.00 (m, 1H, H6), 1.95-1.69 (m, 5H), 1.65-1.45 (m, 2H), 1.35-1.20 (m, 2H), 1.15-1.07 (m, 1H), 1.00 (d, 3H, J = 7.3 Hz, C$\underline{H}_3$6), 0.90 (s, 9H, SiC(C$\underline{H}_3$)$_3$), 0.85 (d, 3H, J = 7.0 Hz, C$\underline{H}_3$2), 0.09 (s, 3H, SiC$\underline{H}_3$), 0.08 (s, 3H, SiC$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.5 (C15), 132.7, 131.6, 76.3, 63.6, 41.6, 40.0, 39.3, 39.0, 37.3, 36.7, 33.3, 32.3, 32.1, 27.50, 27.46, 25.7, 23.71, 23.66, 18.2, 15.0, -4.8; HRMS (EI) calcd for $C_{25}H_{44}O_3Si$ 420.3060, found 420.3046 (M^+)



(4*R*,6*R*)-4-(*tert*-Butyldimethylsilyloxy)-6-(2-((2*R*,3*R*,3a*R*,6*R*,7a*S*)-2,6-dimethyldecahydronaptho[1,2-*b*]oxiren-3-yl)ethyl)tetrahydro-2*H*-pyran-2-one (100 and 101).

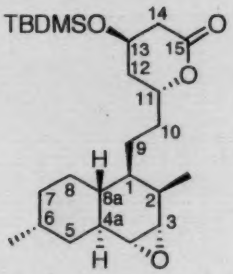
The epoxidation procedure which was followed was the same as that used for the preparation of TBDPS epoxides **89** and **90**. From TBDMS DHL **100a** (2.6×10^{-4} mol, 95.2 mg) the products TBDMS epoxides **100** and **101** (2.3×10^{-4} mol, 101 mg) were obtained as a white solid in a yield of 89%. Analysis of the ^{1}H NMR spectrum revealed that the ratio of α:β epoxide was 3:2. It was found that the

stereoisomers could be separated by flash chromatography (SiO_2, isocratic elution, 2% CH_3CN:CH_2Cl_2). The separation was performed by Mr. Jesse Li.



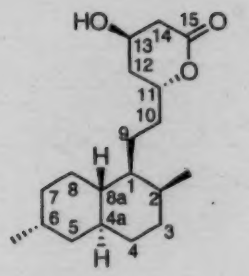
(4*R*,6*R*)-4-(*tert*-Butyldimethylsilyloxy)-6-(2-((1a*R*,2*R*,3*R*,3a*R*,6*R*,7a*S*,7b*S*)-2,6-dimethyldecahydronaptho[1,2-*b*]oxiren-3-yl)ethyl)tetrahydro-2*H*-pyran-2-one (100).

R_f 0.32 (2% CH_3CN:CH_2Cl_2); $[\alpha]_D^{25}$ 52.0° (*c* 0.18, $CHCl_3$); IR ($CHCl_3$ cast) 2954, 2925, 2854, 1742 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.69-4.62 (m, 1H, H11), 4.32-4.28 (m, 1H, H13), 3.19 (dd, 1H, *J* = 4.0, 5.7 Hz, H3), 2.94 (d, 1H, *J* = 4.0 Hz, H4), 2.62 (dd, 1H, *J* = 4.0, 17.4 Hz, H14), 2.56 (ddd, 1H, *J* = 1.7, 4.0, 17.4 Hz, H14'), 2.20-2.14 (m, 1H, H2), 2.10-2.04 (m, 1H, H6), 1.85-1.80 (m, 1H, H12'), 1.79-1.60 (m, 4H), 1.55-1.40 (m, 7H), 1.26-1.14 (m, 2H), 1.08-0.90 (m, 1H, H8a), 1.00 (d, H3, *J* = 7.2 Hz, C$\underline{H}_3$6), 0.95 (d, 3H, *J* = 7.1 Hz, C$\underline{H}_3$2), 0.89 (s, 9H, SiC(C$\underline{H}_3$)$_3$), 0.084 (s, 3H, SiC$\underline{H}_3$), 0.076 (s, 3H, SiC$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.4, 76.0, 63.6, 58.2, 57.0, 41.7, 39.3, 37.7, 36.5, 35.6, 34.6, 33.3, 31.7, 29.0, 27.0, 25.7, 23.3, 22.7, 18.0, 9.6; HRMS (EI) calcd for $C_{25}H_{44}SiO_4$ 436.3009, found 436.3000 (M^+)



(4*R*,6*R*)-4-(*tert*-Butyldimethylsilyloxy)-6-(2-((1a*S*,2*R*,3*R*,3a*R*,6*R*,7a*S*,7b*R*)-2,6-dimethyldecahydronaptho[1,2-*b*]oxiren-3-yl)ethyl)tetrahydro-2*H*-pyran-2-one (101).

R_f 0.40 (2%CH_3CN:CH_2Cl_2); $[\alpha]_D^{25}$ 63.6° (*c* 0.44, $CHCl_3$); IR ($CHCl_3$ cast) 2955, 2926, 2854, 1740 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 4.63 (dddd, 1H, *J* = 3.4, 7.7, 7.7, 7.8 Hz, H11), 4.29 (dddd, 1H, *J* = 3.6, 3.6, 3.6, 3.6 Hz, H13), 3.00 (d, 1H, *J* = 3.1 Hz, H3), 2.75 (d, 1H, *J* = 3.9 Hz, H4), 2.61 (dd, 1H, *J* = 4.4, 17.3 Hz, H14), 2.56 (ddd, 1H, *J* = 1.6, 3.4, 17.3 Hz, H14'), 2.36-2.30 (m, 1H), 2.11-2.05 (m, 1H), 1.85-1.82 (m, 1H), 1.75-1.47 (m, 6H), 1.46-1.35 (m, 2H), 1.32-1.25 (m, 4H), 1.02-0.95 (m, 1H), 1.00 (d, 3H, *J* = 7.1 Hz), 0.92 (d, 3H, *J* = 7.3 Hz), 0.89 (s, 9H, $SiC(C\underline{H}_3)_3$), 0.081 (s, 3H, $SiC\underline{H}_3$), 0.075 (s, 3H, $SiC\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.5, 76.4, 63.6, 58.8, 57.5, 39.3, 38.4, 37.4, 36.8, 36.4, 36.2, 33.2, 32.1, 30.4, 27.6, 25.7, 23.9, 23.8, 18.1, 10.3; HRMS (EI) calcd for $C_{25}H_{44}O_4Si$ 436.3009, found 436.3004 (M^+)

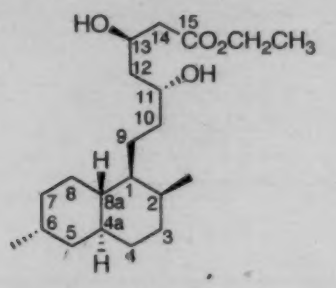


(4*R*,6*R*)-6-(2-((1*S*,2*S*,4a*S*,6*R*,8a*S*)-2,6-Dimethyldecahydronapthalen-1-yl)ethyl)-4-hydroxytetrahydro-2*H*-pyran-2-one (102).

Into a 25 mL round bottom flask with a magnetic stirrer bar was placed DHL **13** (2.24×10^{-4} mol, 68.6 mg), 95% ethanol (10 mL) and palladium on carbon (18.5 mg). The flask was fitted with a rubber septum and a balloon inflated with hydrogen gas. The reaction mixture was stirred overnight at room temperature. The flask was then flushed briefly with Ar and filtered through a pad of Celite. The Celite was then washed with 95% ethanol (3x20 mL) and the solvent was removed *in vacuo*. This provided THL **102** (2.24×10^{-4} mol, 69 mg) as white solid in quantitative yield.

R_f 0.21 (40% EtOAc:hexanes); $[\alpha]_D^{25}$ -118.0° (*c* 0.11, $CHCl_3$); IR (microscope) 2960, 2927, 1664 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.70-4.64 (m, 1H), 4.42-4.39 (m, 1H), 2.76 (dd, 1H, *J* = 5.2, 17.6 Hz), 2.62 (ddd, 1H, *J* = 1.7, 3.9, 17.6 Hz), 1.98-1.89 (m, 4H), 1.82-1.75 (m, 2H), 1.66-1.42 (m, 5H), 1.36-1.10 (m, 7H), 0.96 (d, 3H, *J* = 7.1 Hz), 0.92-0.82 (m, 2H), 0.83 (d, 3H, *J* = 7.1 Hz), 0.78-0.70 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.3, 77.1, 76.0, 62.9, 44.6, 41.9, 39.9,

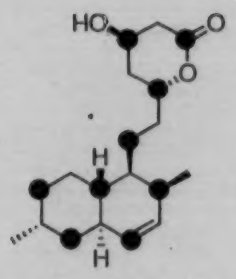
38.6, 37.1, 36.1, 33.2, 32.1, 29.6, 28.4, 27.3, 24.7, 24.3, 18.1, 12.2; HRMS (EI) calcd for $C_{19}H_{32}O_3$ 308.2351, found 308.2344 (M^+)



(3*R*,5*R*)-Ethyl 7-((1*S*,2*S*,4a*S*,6*R*,8a*S*)-2,6-dimethyldecahydronapthalen-1-yl)-3,5-dihydroxyheptanoate (103).

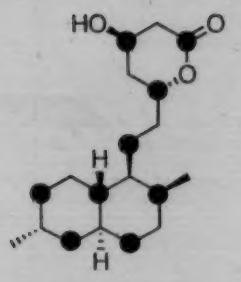
Formed after storage of THL **102**. The excess ethanol in the sample presumably caused the production of the THL ethyl ester **103**.

R_f 0.38 (40% EtOAc:hexanes); $[\alpha]_D^{25}$ 4.90° (*c* 0.30, $CHCl_3$); IR ($CHCl_3$ cast) 3407, 2921, 2854, 1713 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 4.30-4.26 (m, 1H, H13), 4.18 (q, 2H, *J* = 7.2 Hz, $OC\underline{H}_2CH_3$), 3.86-3.85 (m, 1H, H11), 2.49 (d, 1H, *J* = 1.2 Hz, H14), 2.48 (s, 1H, H14), 1.97-1.89 (m, 2H, H2,H6), 1.64-1.44 (m, 9H), 1.34 (ddd, 1H, *J* = 3.0, 4.8, 12.3 Hz), 1.28 (t, 3H, *J* = 7.2 Hz, $OCH_2C\underline{H}_3$), 1.30-1.05 (m, 7H), 1.01-0.94 (m, 1H), 0.96 (d, 3H, *J* = 7.3 Hz, $C\underline{H}_36$), 0.91-0.84 (m, 1H), 0.82 (d, 3H, *J* = 7.1 Hz, $C\underline{H}_32$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.5 (C15), 72.5 (C13), 69.0 ($O\underline{C}H_2CH_3$), 60.7 (C11), 44.7, 42.3 (C14), 42.0, 41.6, 39.9, 37.1, 35.3, 33.2, 32.1, 29.6 (C2), 28.4, 27.3 (C6), 24.9, 24.3, 18.1 ($\underline{C}H_36$), 12.2 ($\underline{C}H_32$); HRMS (+ES) calcd for $C_{21}H_{38}O_4Na$ 377.2662, found 377.2663 (M^++Na)



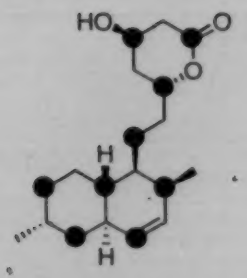
^{14}C-(4*R*,6*R*)-6-(2-((1*S*,2*S*,4a*R*,6*R*,8a*S*)-2,6-Dimethyl-1,2,4a,5,6,7,8,8a-octahydronapthalen-1-yl)ethyl)-4-hydroxytetrahydro-2*H*-pyran-2-one (104).[100, 120]

^{14}C-Dihydromonacolin L **104** was produced using the same procedure as for unlabeled DHL **13** with some modifications. Labeled sodium acetate (1-^{14}C, 250 μCi) was dissolved in sterile, deionized water (5 mL) and dispensed into the production media via a syringe pump in addition to unlabeled sodium acetate (1 g per day) for five days. The crude, labeled DHL was purified by preparative TLC (20 cmx20 cm, 1000 micron SiO_2, eluted twice, 50% EtOAc:hexanes). This provided ^{14}C-DHL **104** (26.1 mg) as a white solid with a total activity of 2.32 μCi. The spectral data matched those obtained for unlabeled DHL **13**.



$^{13/14}$C-(4*R*,6*R*)-6-(2-((1*S*,2*S*,4a*S*,6*R*,8a*S*)-2,6-dimethyldecahydronapthalen-1-yl)ethyl)-4-hydroxytetrahydro-2*H*-pyran-2-one (105).

The labeled $^{13/14}$C-THL **105** was prepared in the same manner as that for unlabeled THL **102**. The amounts of reagents used were ^{14}C-DHL **104** (1.70×10^{-5} mol, 5.2 mg, 0.48 μCi), ^{13}C-DHL **106** (6.86×10^{-5} mol, 21 mg), palladium on carbon (10 mg) and 95% EtOH (3 mL). After purification, $^{13/14}$C-THL **105** (8.50x10-5 mol, 26.4 mg, 0.43 μCi) was obtained as a white solid in quantitative yield. The spectral data for labeled $^{13/14}$C-THL **105** matched those obtained for unlabeled THL **102**.



^{13}C-(4*R*,6*R*)-6-(2-((1*S*,2*S*,4a*R*,6*R*,8a*S*)-2,6-Dimethyl-1,2,4a,5,6,7,8,8a-octahydronapthalen-1-yl)ethyl)-4-hydroxytetrahydro-2*H*-pyran-2-one (106).

^{13}C-dihydromonacolin L (**106**) was produced using the same procedure as for unlabeled DHL **13**. The 1-^{13}C-sodium acetate (1g) was fed once per day for five days. The amount of ^{13}C-DHL **106** obtained was the same as for unlabeled DHL **13**. The spectral data matched those obtained for DHL **13**.

Procedure for biotransformation of $^{13/14}$C-tetrahydromonacolin L 105

The procedure followed was the same as that for the preparation of lovastatin (**1**). A solution of $^{13/14}$C-THL **105** (31 mg, 0.43 μCi in 5 mL of ethanol) was delivered to the fermentation of *A. terreus lovc* over one day via syringe pump (1 mL per 4 hours). After purification by preparative TLC, $^{13/14}$C-THL **105** was recovered with a total activity of 0.31 μCi.

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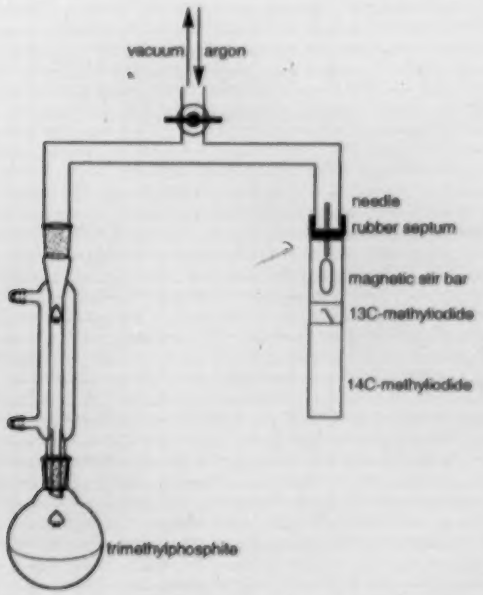
Appendix A

Method for the transfer of ^{14}C-methyl iodide from a break seal ampoule to reaction flask



The procedure used was a combination of Method A and Method B in the Amersham document.[134]

1. The apparatus was assembled as shown without reagents and the glassware (excluding the ampoule) was flame dried under Ar.

2. The entire apparatus was evacuated and then flushed with Ar three times.

3. Trimethyl phosphite was added to the reaction flask via syringe and positive pressure of Ar. The ^{13}C-methyl iodide was introduced to the top of the break seal ampoule by inserting a needle through the side wall of the rubber septum.

4. The bottom of the ampoule was cooled to -78 °C (dry ice: acetone).

5. Then the seal was broken by raising the stirrer bar using a magnet and allowing the stir bar to fall.

6. The reaction flask was cooled to -78 °C and the apparatus was evacuated briefly by applying the vacuum and the stopcock was then closed.

7. The contents of the ampoule were then distilled over to the reaction flask by gently warming the ampoule with a heat gun.

8. The water condenser was then cooled, the apparatus was open to the Ar line and the reaction flask was placed in a 40 °C oil bath to initiate the Arbuzov reaction.